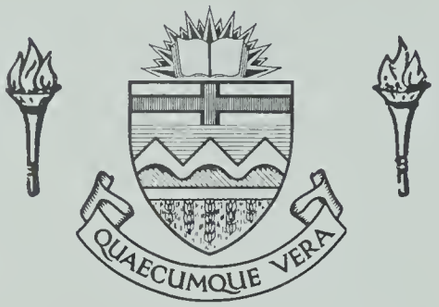

THE UNIVERSITY OF ALBERTA

CHEMICAL CONSTITUENTS OF SOME NATIVE MEDICINAL PLANTS

by

© LOIS MARGARET BROWNE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

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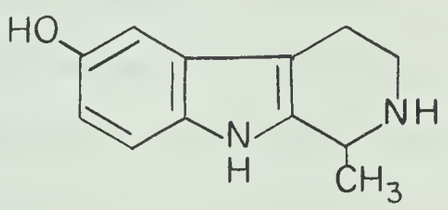
FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "CHEMICAL CONSTITUENTS OF SOME NATIVE MEDICINAL PLANTS", submitted by Lois M. Browne, in partial fulfillment of the requirements for the degree of Master of Science.

ABSTRACT

Shepherdia argentea (buffaloberry) is a plant native to Alberta which was used in the folk medicine of the Blackfoot. We have investigated the alkaloidal components of both *Shepherdia argentea* and *Shepherdia canadensis*. *Shepherdia argentea* contains several simple amines and the alkaloid tetrahydroharmol. On the other hand, *Shepherdia canadensis* contains tetrahydroharmol, serotonin and a new alkaloid, shepherdine (see below).



Clematis ligusticifolia was also used medicinally by the Blackfoot. We have investigated *Clematis ligusticifolia* and have found β-sitosterol and 4,7-dimethoxy-5-methyl-coumarin to be present.

ACKNOWLEDGEMENTS

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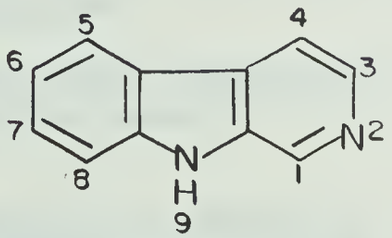
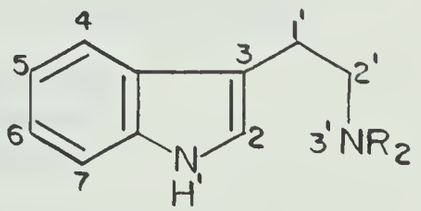
INTRODUCTION

An Indian writer has said, "You take the root of a cherry tree, boil it, and strain it through a cloth and you can use it as a cure for diarrhea". The tree mentioned was probably buffalo or bullberry, Shepherdia argentea.[1]

Two species of Shepherdia are indigenous to Alberta: Shepherdia argentea Nutt., a thorny shrub common around coulees and sloughs in the southern area, and Shepherdia canadensis (L.) Nutt., an unarmed under shrub common in the Cypress hills, in wooded places and along river banks.[2] The plant material used in our work was collected near Lethbridge, the University of Alberta Experimental Farm, and in the south Edmonton area.

The bases isolated from Shepherdia are β-carbolines (harmala alkaloids) and tryptamines.





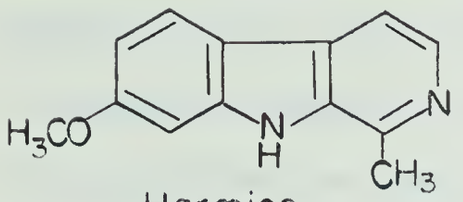
The harmala alkaloids were first found in Peganum harmala but have since been found in plants of many families (See table 1). The chemistry of the harmala alkaloids has

PLANTS AND THEIR CONTAINED ALKALOIDS[5]

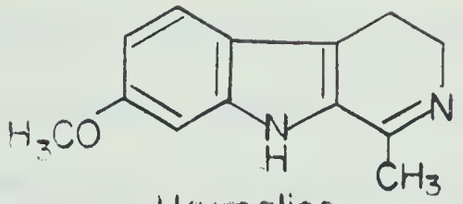
PLANT AND FAMILY	ALKALOID
Peganum harmala L. Zygophyllaceae (Rutaceae)	Harmine Harmaline Harmalol Harmidine
Symplocos racemosa Roxb. Symplocaceae (Styracaceae)	Harman (Loturine)
Sickingia rubra K. Schum. (Arariba rubra Mart.) Rubiaceae	Harmine (Aribine)
Eleagnus angustifolia L. Eleagnaceae	Tetrahydroharman Tetrahydroharmol N-Methyltetrahydroharmol
Banisteria caapi Spruce Malpighiaceae	Harmine Harmaline Tetrahydroharmine
Cabi paraensis Ducke Malpighiaceae	Harmine
Banisteriopsis inebrians Morton Malpighiaceae	Harmine
Leptactina densiflora Hook. f. Rubiaceae	Tetrahydroharmine Tetrahydroharman
Passiflora incarnata L. Passifloraceae	Harmine Harman Harmol
Zygophyllum fabago L. Zygophyllaceae (first isolation of harmol)	Harmine Harman Harmol
Arthrophytum leptocladum Popov (Chenopodiaceae)	4-Methyl- and 3,4-dimethyl-3,4,5,6-tetrahydro-4-carboline
Petalostylis labicheoides R. Br. (Leguminosae)	Tetrahydroharman

Table 1.

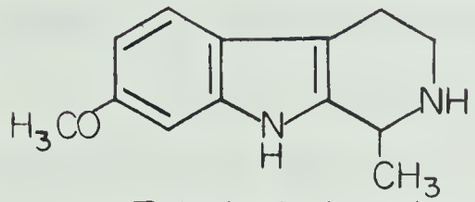
Harmala Alkaloids



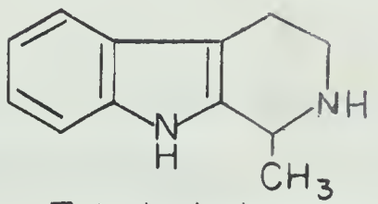
Harmine



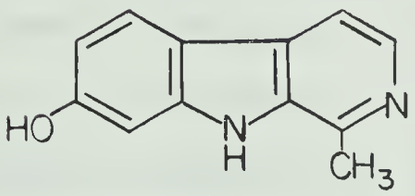
Harmaline



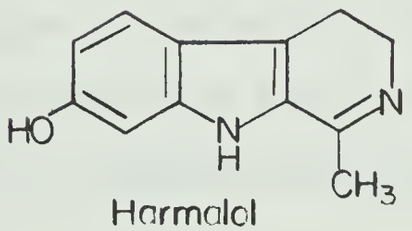
Tetrahydroharmine



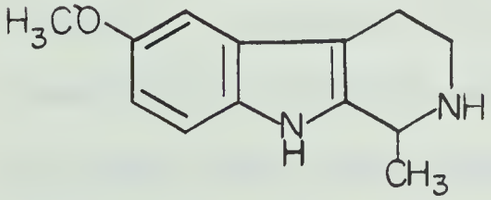
Tetrahydroharman



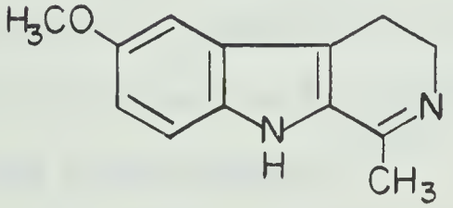
Harmol



Harmalol



6-methoxytetrahydroharman



6-methoxyharmalan

Figure A.

been reviewed by Henry,[3] Marion[4] and Manske.[5] In general, the harmala alkaloids crystallize readily having high melting points. Although the tetrahydro bases have one asymmetric center, only one case of optical activity is known; tetrahydroharmine mp 199 - 200°C, $[\alpha]_D$+32. Bases in which the pyridine nucleus is at least partially dehydrogenated show strong fluroescence in ultraviolet light, e.g., harmine fluoresces indigo blue in acid solution becoming yellow-green in alkaline solution.

Plants containing harmala alkaloids (β-carbolines) have been in use for a long time. Peganum harmala, the seeds of which contain harmine, harmaline and harmalol has been used throughout the Middle East both as a spice and as an intoxicant. Its medical and psychotropic properties are known in India where it has been used as an anthelmintic. As well, a species of Banisteriopsis which is a source of harmala alkaloids is used as a snuff by Indians of South America.

Recently, substances closely related to the harmala alkaloids have been discovered in animals. Adrenoglomerulotropine, a hormone of the pineal gland, is identical with 6-methoxytetrahydroharman and has been shown to be formed *in vivo* from 5-methoxytryptamine and acetaldehyde.[6a,b] Another substance, 6-methoxyharmalan has been shown to be derived from melatonin.[7]

The most extensive pharmacological investigation of the harmala alkaloids was carried out by Gunn and coworkers.[8]

They showed a relationship between the chemical structure and the pharmacological activity of the various alkaloids of the harmala group. Harmine in large doses causes tremors and clonic convulsions presumably by action on the cerebral cortex. Lethal doses cause convulsions followed by motor paralysis (due to the depressant action on the central nervous system), paralyze respiration and produce a fall in temperature in mammals. Harmine produces a fall in blood pressure due to weakening of the cardiac muscle and depresses contractions of smooth muscle, except for the uterus which it causes to contract powerfully especially in the rabbit. Harmine is more toxic than quinine to most protozoa and has been shown to be a monoamine oxidase (MAO) inhibitor in the rat.[9] Harmine is reported to be an hallucinogen.[10]

Reduction of harmine to harmaline (dihydroharmine) or tetrahydroharmine does not greatly alter the pharmacological activity, but gives quantitative differences. The relative minimum lethal dose (MLD) is as follows: harmine 2, harmaline 1, tetrahydroharmine 3.

Removal of the methoxyl group does not seem to cause any significant differences in pharmacological activity. Tetrahydroharman has all the characteristics of harmine, being slightly less active than tetrahydroharmine.

Substituting a hydroxyl group for the methoxyl group shows some change pharmacologically. Harmol gives no clonic convulsions but a progressive paralysis of the central

nervous system. It has been suggested that the hydroxyl group is concerned with suppression of convulsions since both harmine and tetrahydroharmine cause convulsions. Harmol has a diminished toxic action on protozoa but causes increased stimulation of the uterus.

Harmalol (dihydroharmol) has the same pharmacological activity as harmol, differing only quantitatively. It is a powerful uterine stimulant (ecbolic) showing 17 times the activity of quinine with one-half the toxicity in the rabbit.

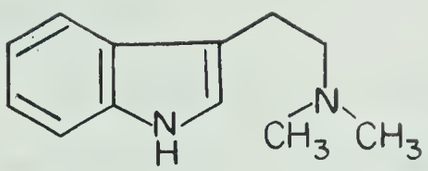
The pharmacological properties of the harmala alkaloids form the basis of their therapeutic use. Harmine has gained a reputation for relieving rigidity of muscles in post-encephalitic conditions and has been shown to cause clinical improvement in cases of Parkinson syndrome.[11] Harmine produces a fall in temperature in mammals and recently, a patent for an analgesic containing harmine has been applied for in the USA.[12] Synthetic ethers of harmol, especially O-n-amylharmol, have been used clinically in the treatment of angina pectoris but their therapeutic value in such cases has not been unequivocably established. Harmalol, shown to be the most effective ecbolic in animals, gives less effect on the human uterus. The therapeutic value of harmalol as an ecbolic is questionable. Malaria has been treated with harmine and/or harmaline which were found to be 50% effective. The therapeutic value of harmine

or harmaline in malaria has not been adequately shown. Harmine has an anthelmintic effect on nematodes and cestodes.[13] As well, synthetic ethers of harmol (n-nonylharmol being most efficient) find use in treatment of amebic dysentry.[14] There is therapeutic interest in drugs which have been shown to inhibit the enzyme monoamine oxidase in animals. Harmaline, shown to be a potent inhibitor in animals is a short acting inhibitor in man.[15]

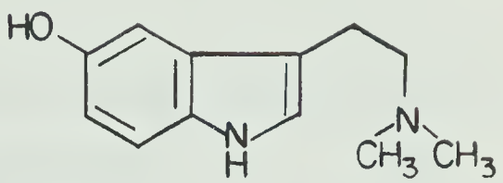
The harmala alkaloids qualitatively show the same psychoactivity as harmaline. Harmaline, a hallucinogen, acts as a stimulant on the mid brain reticular formation while its action on the brain cortex is more depressant. This is suggested by the vividness of imagery, the phenomenon of double images and persistance of images when under the influence of harmaline. The neurophysiological picture matches the traditional yagé dreaming: the state described involving lethargy, immobility, closed eyes, generalized withdrawal from the environment but at the same time alertness of mental processes and activation of fantasy.[16]

Tryptamines are widely distributed in the plant kingdom, being present in edible fruits (e.g. the banana, tomato, plum, eggplant) and in fungi belonging to the genus Panaeolus[17] as well as other plants. Recently, their chemistry has been reviewed by Marion[18] and by Saxton.[19] Several of the naturally occurring tryptamine derivatives exhibit psychotomimetic activity. N,N-dimethyltryptamine

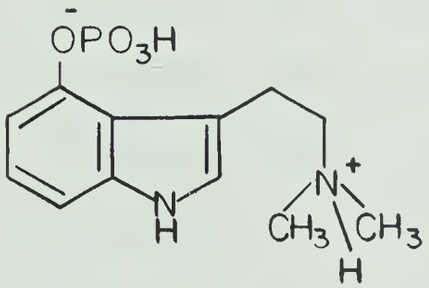
Tryptamines



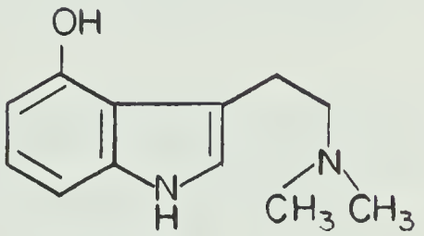
N,N-dimethyltryptamine



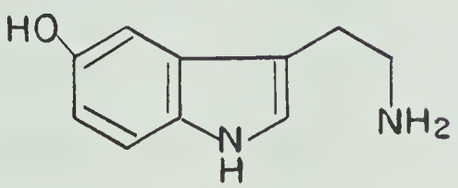
Bufotenine



Psilocybin



Psilocin



Serotonin

Figure B.

and/or bufotenine have been shown to be the active constituent(s) in the narcotic snuff prepared from plants by certain American Indian tribes,[20] while psilocin and psilocybin occur in various fungi which are reported to be hallucinogenic.

Serotonin, on the other hand has been shown to be the active irritant in cowhage (Mucuna pruriens D.C.)[21] and stinging nettle (Urtica dioica).[22] In mammals, serotonin is found in the brain, in the blood and in the tissues of the stomach, intestines and lungs; its function in these sites has not yet been fully eludicated. There is little doubt that it plays an extremely important role in the central nervous system especially in the brain.

5-Methoxytryptamines as well as 5-hydroxytryptamines and β-carbolines have been found to be present in South American snuffs used for intoxicating purposes. The β-carbolines are monoamine oxidase inhibitors and could potentiate the action of the simple indoles, thus the combination of β-carbolines and tryptamines could be advantageous.

The hallucinogenic activity of the tryptamines varies somewhat from that of the β-carbolines. The neurophysiological state is described as headache, salivation, vomiting, profuse perspiration, unsteady gait and a typical facial expression, as well as macroptic illusions possibly resulting from disturbances in the peripheral motor perception.[23]

Another native plant with reported medicinal value

is Virgin's bower or Clematis. Two species of Clematis are indigenous to Alberta: Clematis columbiana (Nutt.) T & G, a climbing plant found in shady woodland of the Cypress hills and the Foothills; and Clematis ligusticifolia (Nutt.) a climbing plant common in coulees and ravines in southern Alberta. The plant material used in our work was supplied by A. Johnston of the Lethbridge area.

Several components of other species of Clematis have been reported. β-Sitosterol, linoleic acid, oleic acid, and myristic acid have been isolated from Clematis angusticifolia.[24] Clematoside C, an oligoside of oleanolic acid has been isolated from Clematis mandshurica,[25] while terniflorin (apigenin-7-(p-coumaroyl)-β-Dglucoside) has been isolated from Clematis terniflora.[26] Two substances thought to be responsible for the antimicrobial activity of Clematis dioscoreifolia have been shown to be anemonin and protoanemonin.[27] A medicinal preparation from Clematis roots used in the USSR contains a coumarin derivative, the structure of which was not reported.[28]

β-Sitosterol and a coumarin were isolated from the neutral material of Clematis ligusticifolia. Coumarin derivatives all have qualitatively the same action in the body, with one major pharmacological action; inhibition of blood-clotting mechanism. Coumarins act by diminishing the plasma prothrombin time due to interference with the normal synthesis of clotting factors in the liver. The

basic mechanism of inhibition involves interference with the action of Vitamin K, probably by competition of the coumarin with Vitamin K for an enzyme essential to prothrombin synthesis. Coumarins find therapeutic value as anticoagulants in treatment of myocardial infarction, angina pectoris, rheumatic heart disease, venous thrombosis and pulmonary embolism.[29]

This thesis describes work which has led to the isolation and elucidation of structure of the alkaloids of Shepherdia argentea and Shepherdia canadensis. As well, the isolation and elucidation of some neutral components of Clematis ligusticifolia is described.

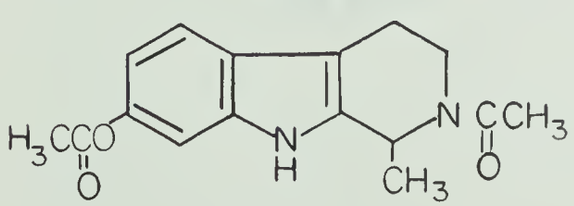
DISCUSSION AND RESULTS

ISOLATION AND STRUCTURE DETERMINATION OF THE ALKALOIDS OF SHEPHERDIA ARGENTEA AND SHEPHERDIA CANADENSIS

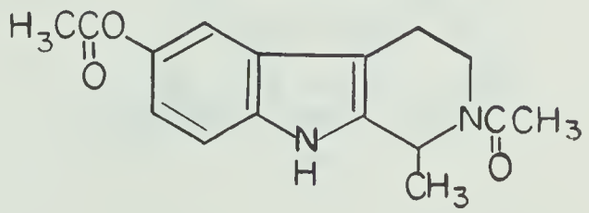
Our interest in the medicinal value of Shepherdia argentea[1] led to its investigation first. Extraction of dried, ground plant, followed by removal of the acidic and neutral materials gave the crude bases. Since the crude bases darkened on exposure to light and/or air, several methods to facilitate its handling were explored. Spontaneous evaporation of an ethereal solution of crude bases left only a black residue, while attempted preparation of the hydrochloride salts of the crude bases also gave black material which could not be purified by crystallization. Acetylation with acetic anhydride and pyridine gave a yellow-brown foam the TLC of which showed one major component. Acetylation thus appeared to be a good method of handling the basic material and the initial separation was carried out on this material. Elution chromatography on basic alumina (Fisher) led to the recovery of very little material, even with polar solvents (chloroform-methanol, 20:1). On the other hand, elution chromatography on deactivated alumina (Woelm alumina, activity 3) gave two main fractions: a mixture of non-polar acetylated bases, and a major, more polar fraction which appeared (TLC) to be a single component.

The major fraction, eluted with benzene-chloroform (4:1) crystallized from ethyl acetate. The compound thus obtained, melting point 202-203°C, is optically inactive and has a typical indole chromophore (λ_{max}^{EtOH} 229mμ (ε=21,000) 282mμ(ε=3,500)) in the ultraviolet.[30] The infrared spectrum shows indolic N-H absorption (3450, 3300 cm^{-1}), as well as an ester (1745 cm^{-1}) and a tertiary amide (1625 cm^{-1}).[31] A one proton singlet at τ1.07 in the NMR, which disappears on addition of D_2O, is assigned as the indolic N-H. As well, there is an ABX system of aromatic protons. A one proton quartet at τ4.35, J=7 cps, coupled to a three proton doublet at τ8.66, J=7 cps, indicates a $-CHCH_3$ group. The spectrum also shows an ABCD system of methylene protons at τ6.08 (1H), 6.65 (1H), and 7.34 (2H); and two three proton singlets due to the acetyl of an amide (τ7.73) and an ester (τ7.82) are present.[32] The formula, $C_{16}H_{18}N_2O_3$ (m/e 286), was established by high resolution mass spectrometry. Important fragment ions appear in the mass spectrum at m/e 271, 229, 201, 187.

The evidence presented to this point was consistent with either structure 1 or structure 2.



1



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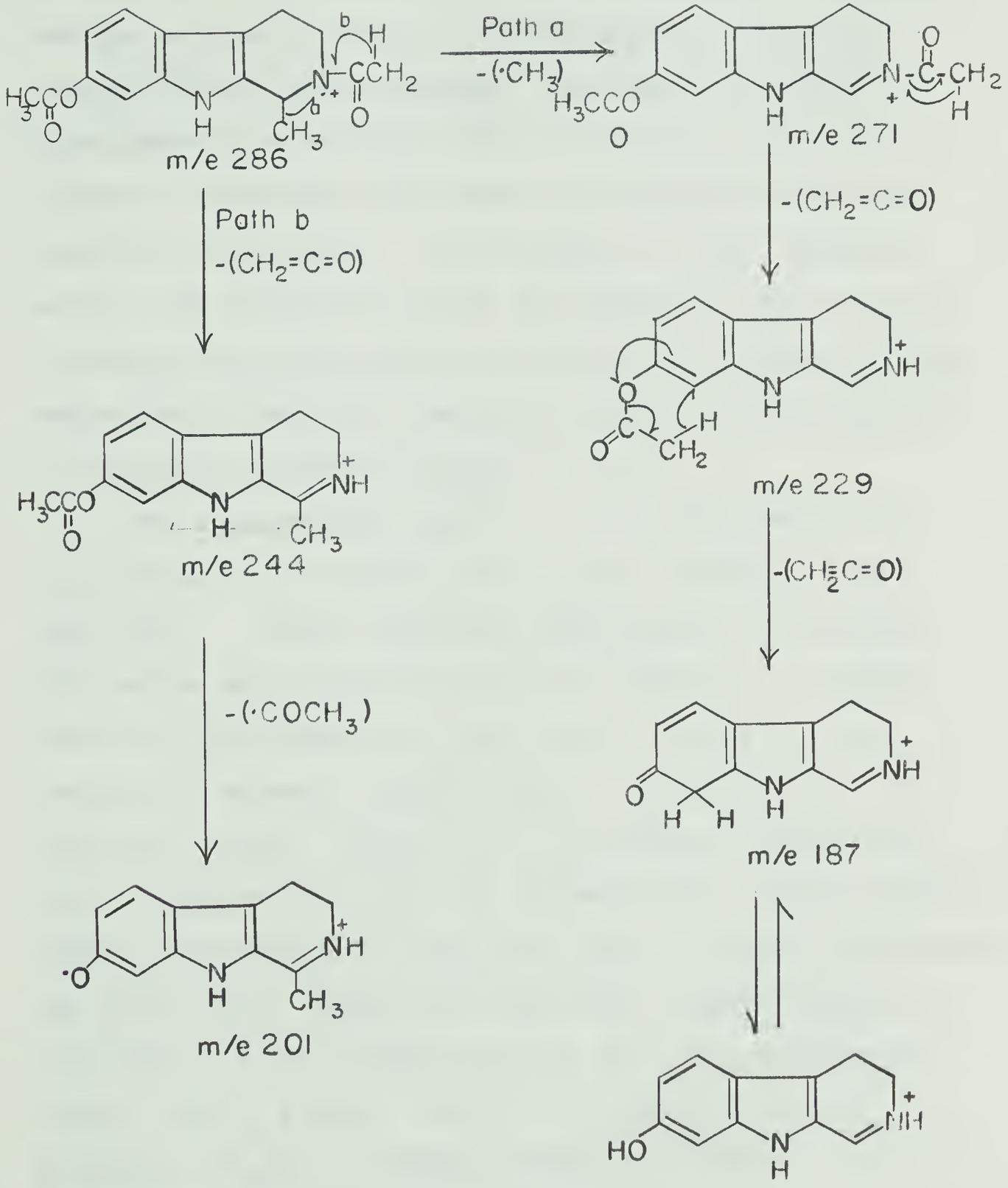
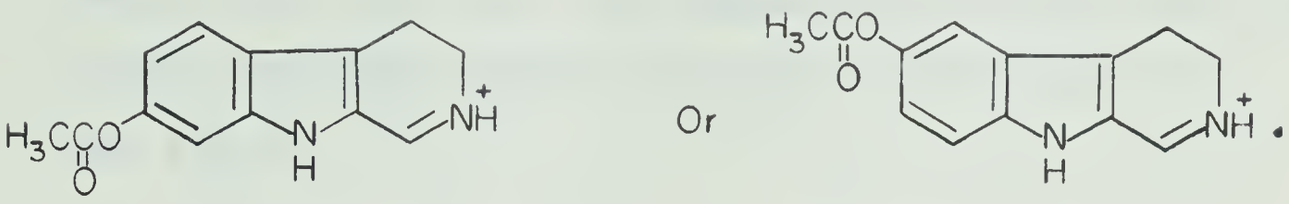

m/e 286
Path a
-(·CH3)
m/e 271
-(CH2=C=O)
Path b
-(CH2=C=O)
m/e 229
m/e 244
-(CH2=C=O)
-(·COCH3)
m/e 187
m/e 201

Figure 1

Both would display the indole N-H, an ABX system characteristic of a 1,2,4-trisubstituted benzene ring, a low field methine coupled to $-CCH_3$, the ABCD system of methylene protons and two acetyl groups. Spin-spin decoupling experiments have verified that the methine proton is coupled to the methyl and that the methylene protons are coupled to one another. On the basis of their chemical shifts, the equatorial proton and the axial proton on the carbon α to the amide nitrogen resonate at τ6.08 and τ6.65, respectively; while the methylene protons on the carbon β to the amide nitrogen resonate at τ7.34.

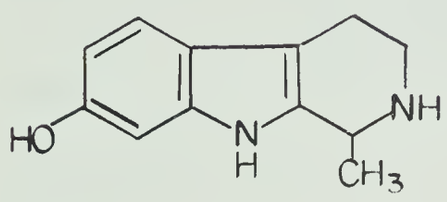
The fragmentation pattern in the mass spectrum of the alkaloid is consistent with either structure 1 or 2 (see fig 1). Indole alkaloids consisting of an aromatic part and an alicyclic part would be expected to fragment mainly by the breaking of bonds in the aliphatic part, leaving the aromatic system intact. The presence of an additional group, $(OCOCH_3)$, in the aromatic system would not be expected to change the fragmentation pattern appreciably, particularly if this group does not greatly influence the stability of either the positively charged species or the radical.[33] The intense peak at m/e 271 indicates the facile loss of a methyl radical, as expected from the grouping $-CCH_3$-N-. Hydrogen transfer followed by loss of ketene gives rise to the base peak, m/e 229, which may be due to the ion



The intense peak at m/e 187 (49%) may arise from the base ion by hydrogen transfer and the resultant loss of ketene from the acetoxy group. In another fragmentation pathway, the loss of ketene from the molecular ion, followed by the loss of an acetyl radical may give rise to the peak at m/e 201. This further supports the presence of an acetoxy group.[34]

To gain further information about the alkaloid, we felt that isolation of the free base was desirable. Elution chromatography of the crude bases on deactivated alumina led to the isolation of an alkaloid. The thin layer chromatographic behavior (alumina, chloroform-methanol (20:1)) suggested it was pure, but attempted crystallization was unsuccessful. Further, the alkaloid discolored when exposed to air and/or light in a solvent for a short time, thus the non-crystalline alkaloid was characterized.

The formula, $C_{12}H_{14}N_2O$ (m/e 202), was established by high resolution mass spectrometry. Other pertinent peaks are m/e 187, 172, 159. The fragmentation pattern was consistent with that expected for either structure 3 or 4. (see fig 2).



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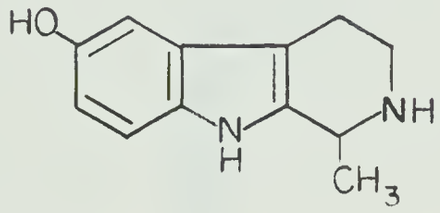


4

Relatively few intense ions suggest an aromatic and aliphatic structure. The loss of 15 mass units to give the base ion at m/e 187 suggests a methyl group on a carbon α to a nitrogen.

The alkaloid tetrahydroharmol (3) had previously been isolated by T. Platanova and co-workers from the bark of Elaegnus angustifolia.[35] Comparison of physical and spectral data with that published for tetrahydroharmol suggested that the free base isolated from S. argentea was tetrahydroharmol. The melting point of the non-crystalline base is 254.5°C (reported 256°C[35]). The UV spectrum (λ_{max}^{EtOH} 229 mμ, log ε = 4.57; 270 mμ, log ε = 3.77; 299 mμ, log ε = 3.85) and $IR_{(nujol)}$ spectrum correspond closely to

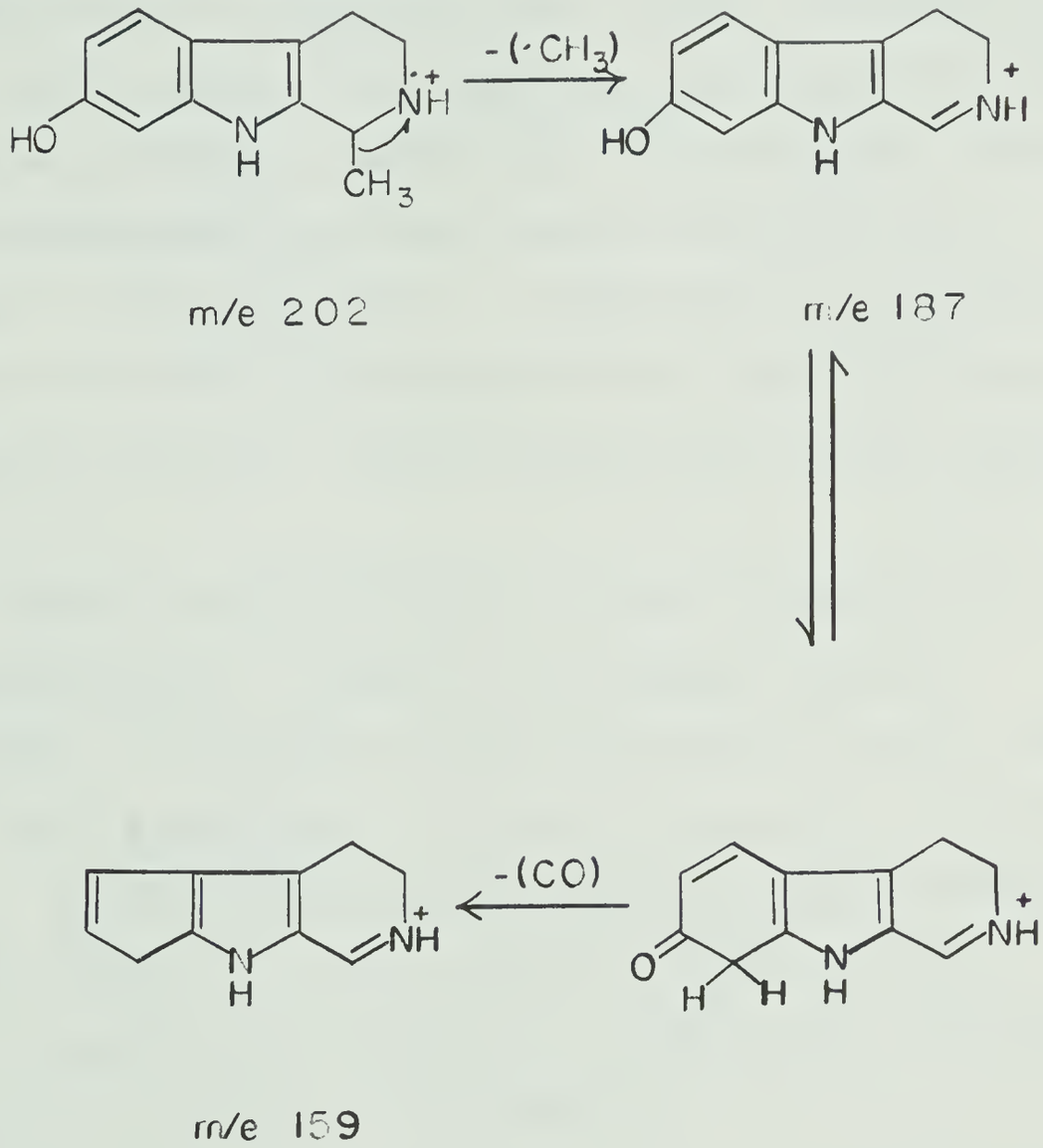


Figure 2

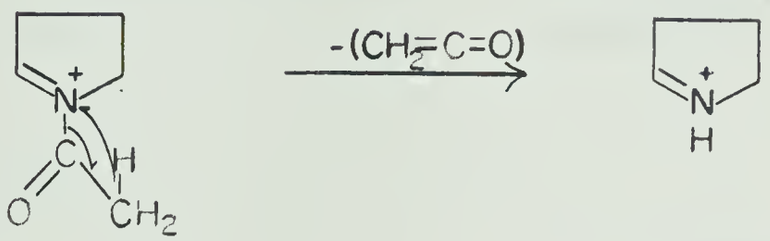
that published for tetrahydroharmol. Finally, the hydrochloride salt of the non-crystalline base melted at 230°C with decomposition (reported 235°C[35]). After standing several months it melted at 235°C without decomposition.

On the basis of the above correlation, we felt that the non-crystalline alkaloid was tetrahydroharmol (3) and the corresponding acetylated alkaloid had structure 1. As will be discussed later, a comparison of a synthetic sample of N,O-diacetyltetrahydroharmol with the acetylated naturally occurring alkaloid showed the two were identical.

Having established that the major alkaloid is tetrahydroharmol, a member of the harmala group of alkaloids, we decided to look at the minor fraction isolated from S. argentea, a mixture of non-polar acetylated bases. Further separation was achieved by gas chromatography using a 10' x 1/4", 5% SE 30 column at 205° C.[36] Seven compounds were isolated and samples were collected for mass spectrometric investigation.

The first of the compounds (5) has a retention time of 6.5 minutes. The molecular formula, $C_6H_{11}NO$ (m/e 113), was established by high resolution mass spectrometry. Important fragment ions appear in the mass spectrum at m/e 85, 71, 70, 44, 43. Compound 5 was identified as N-acetylpyrrolidine on the basis of its mass spectrum. The intense ion, m/e 70 (73%), originates from the M-1

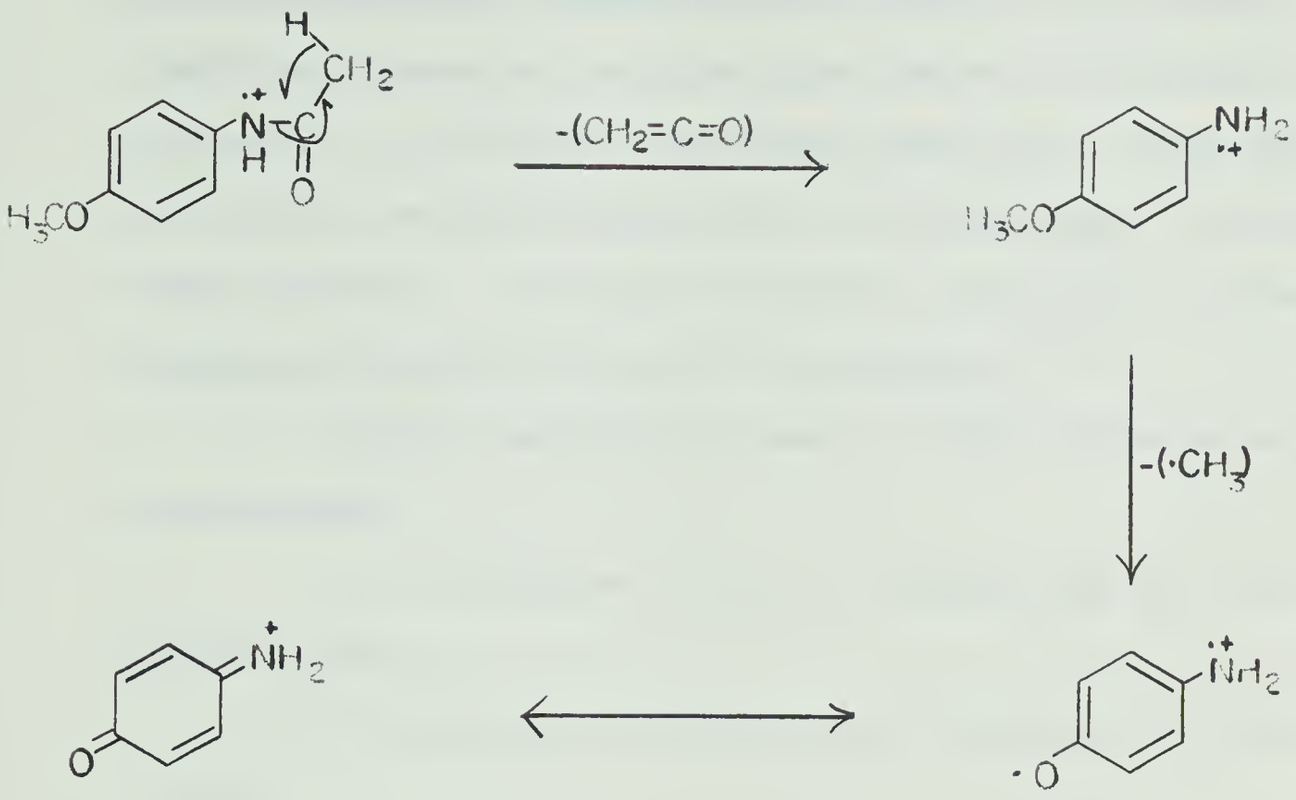
species via hydrogen transfer from the acetyl group and the loss of ketene.[37]



m/e 112 m/e 70

An authentic sample of N-acetylpyrrolidine was prepared by acetylation of pyrrolidine. Purification by vacuum distillation yielded N-acetylpyrrolidine which gave GC retention time and mass spectrum identical with that of compound 5.

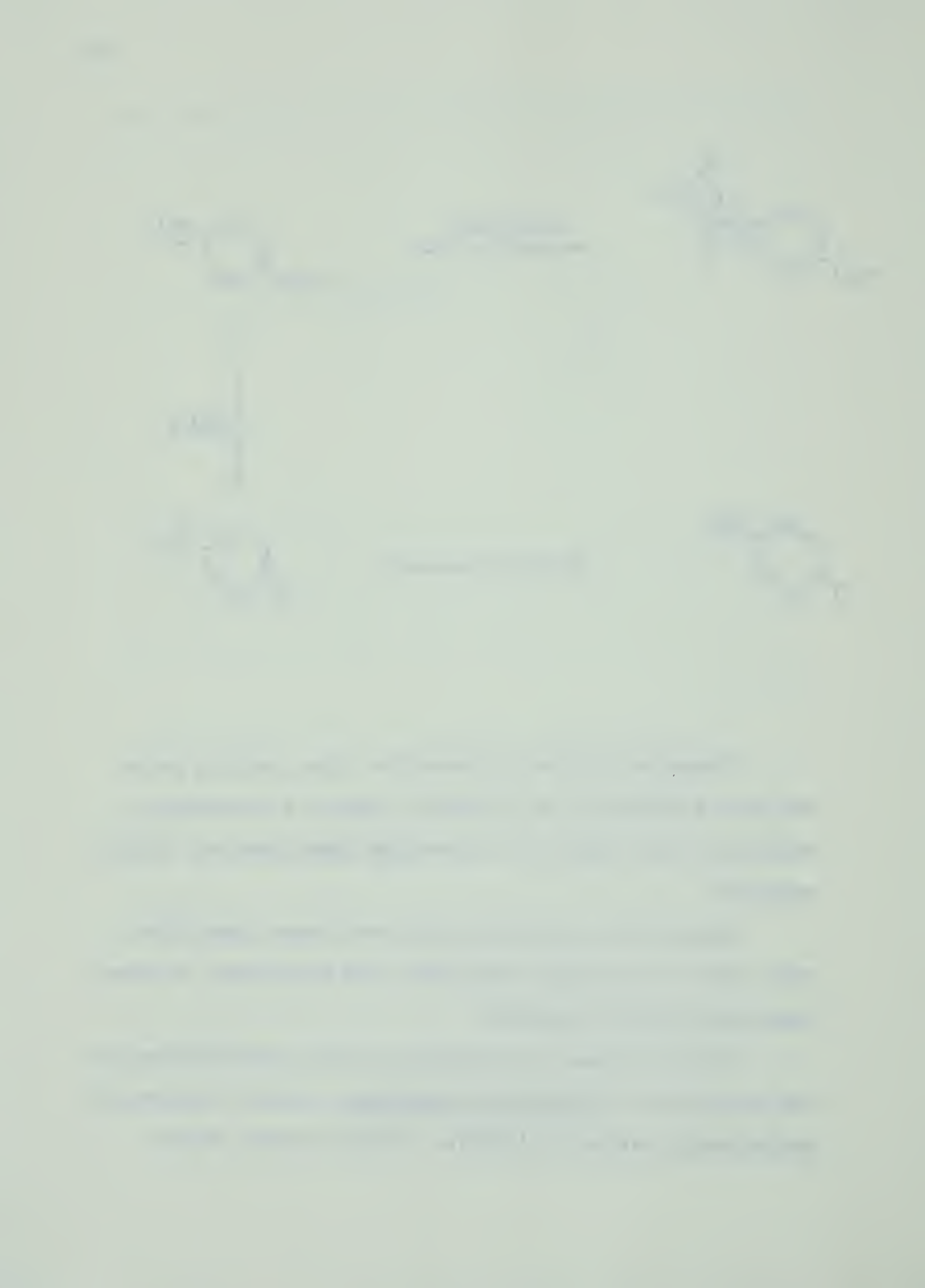
The last of the compounds isolated from the gas chromatograph, compound 6, has a GC retention time of 23 minutes. The molecular formula, $C_9H_{11}NO_2$ (m/e 165), was established by high resolution mass spectrometry. Other fragment ions occur at m/e 123, 108 (base), 95, 80, 65, 43. Compound 6 was identified as N-acetyl-p-anisidine on the basis of its melting point and mass spectrum. The base ion, m/e 108, may arise from the molecular ion via hydrogen transfer from the acetyl group and the resultant loss of ketene to give the fragment ion m/e 123, followed by the loss of a methyl radical.



Comparison of the GC retention time, melting point and mass spectrum of an authentic sample of N-acetyl-p-anisidine with compound 6 showed they were identical in all respects.

Because the non-polar acetylated bases identified were shown to be simple compounds, the structures of those remaining were not pursued.

We then turned our attention to the investigation of the alkaloids of Shepherdia canadensis, another species of buffaloberry native to Alberta. Finely ground, dried



plant was extracted with methanol in a Soxhlet extractor. Excess solvent was removed and the acidic and neutral fractions separated from the crude bases by acid-base extraction. As before, the crude bases were acetylated and further separated by elution chromatography on deactivated alumina. Six major fractions isolated, in order of increasing polarity, were the following:

1) simple acetylated bases (4.5%). These were not investigated.

2) an antioxidant probably originating from silicone grease (0.5%).

3) 7-acetoxy-2-acetyl-1,2,3,4-tetrahydro-2-carboline (0.5%).

4) N,O-Diacetyltetrahydroharmol (4&5 35%).

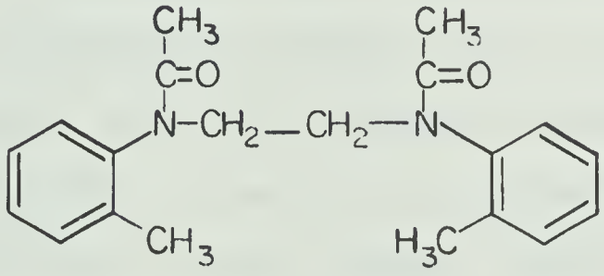
5) N,O-Diacetylshepherdine.

6) N,O-Diacetylserotonin (17%).

The first acetylated base was isolated by elution chromatography on deactivated alumina and was shown to be a pure compound by TLC (alumina, chloroform; alumina, ethylacetate; alumina, dichloromethane-chloroform (1:1)). It crystallizes from acetone (mp 148-150°C) and is optically inactive. It has an aromatic chromophore as shown by the UV spectrum (λ_{max}^{MeOH} 270 mμ). The IR spectrum shows aromatic absorptions at 3050, 1600 and 1495 cm^{-1} as well as carbonyl absorption of a tertiary amide (no N-H stretch, carbonyl absorption at 1665 cm^{-1}). The NMR spectrum has

an A_2B_2 system of eight aromatic protons centered at τ2.74; an AA'BB' system at τ5.63 and 6.83 due to the grouping $-CH_2-CH_2-$; a six proton singlet at τ7.79 due to two acetyl groups and a six proton doublet at τ8.39 due to two methyl groups. No proton exchanged on addition of D_2O indicating that there was no active hydrogen present. The molecular formula, $C_{20}H_{24}N_2O_2$ (m/e 324), was established by high resolution mass spectrometry. Other intense peaks occur in the mass spectrum at m/e 175, 162, 149, 133, 120, 118, 91.

The evidence presented thus far is consistent with the structure 7.



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It would show in the NMR an A_2B_2 system of a 1,2-disubstituted benzene ring, an AA'BB' system of methylene protons, two acetyl groups and two methyl groups. Spin-spin decoupling experiments showed that the methylene groups were coupled to one another, however the methyl groups were not coupled to any proton. In the structure proposed, the

signal assigned to the methyl groups might be split only if rotamers were present. But in such a case, the signal assigned to the acetyl of the amide would also be expected to split. We decided to show the presence or absence of rotameters using an NMR temperature study.

At room temperature in pyridine - $\underline{d}_5$ the NMR spectrum shows a six proton doublet at τ7.97 due to the acetyls of the amides and a six proton doublet at τ8.38 due to the two methyl groups. At 90°C the doublet at τ7.97 becomes a sharp singlet, while the doublet at τ8.38 becomes a broad singlet. Thus increasing the temperature causes the signals to collapse to singlets, confirming the presence of rotamers.

The mass spectral fragmentation pattern of the amide isolated is also consistent with the structure proposed. In the major fragmentation pathway (see fig 3), a McLafferty rearrangement involving hydrogen transfer to the carbonyl and expulsion of an o-tolyl amide ion radical (149 mass units) may result in the intense radical ion at m/e 175. A second rearrangement may then occur, in which there is further hydrogen transfer followed by the loss of ketene to give the radical ion at m/e 133. Finally loss of CH_3,HCN from the latter may give the tropylium ion (m/e 91). In the minor fragmentation pathway, β-fission occurs to give the radical at m/e 162, then hydrogen transfer followed by loss of ketene may result in the fragment ion at m/e 120.

While the evidence presented fits the structure

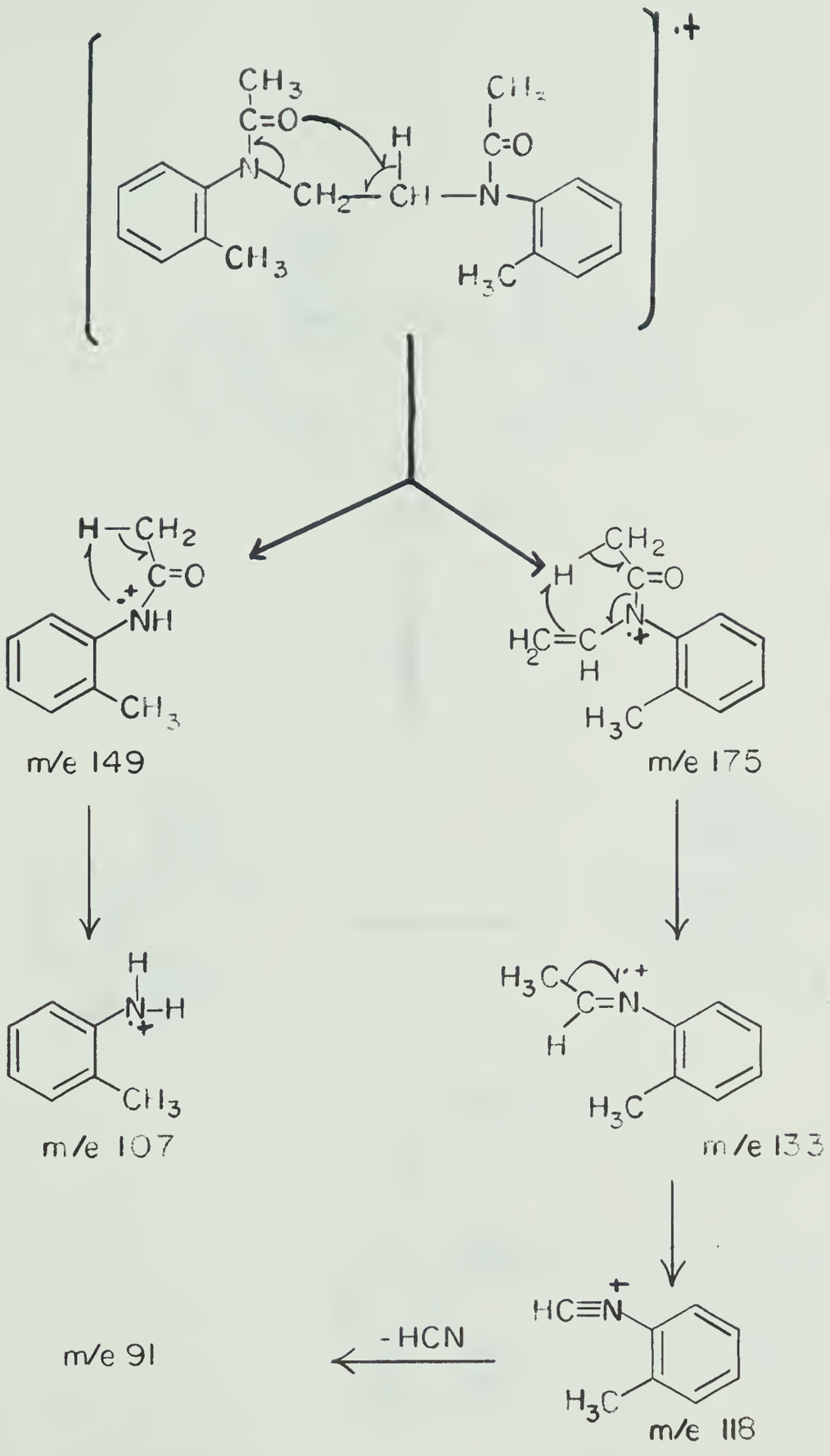


Figure 3 Path a

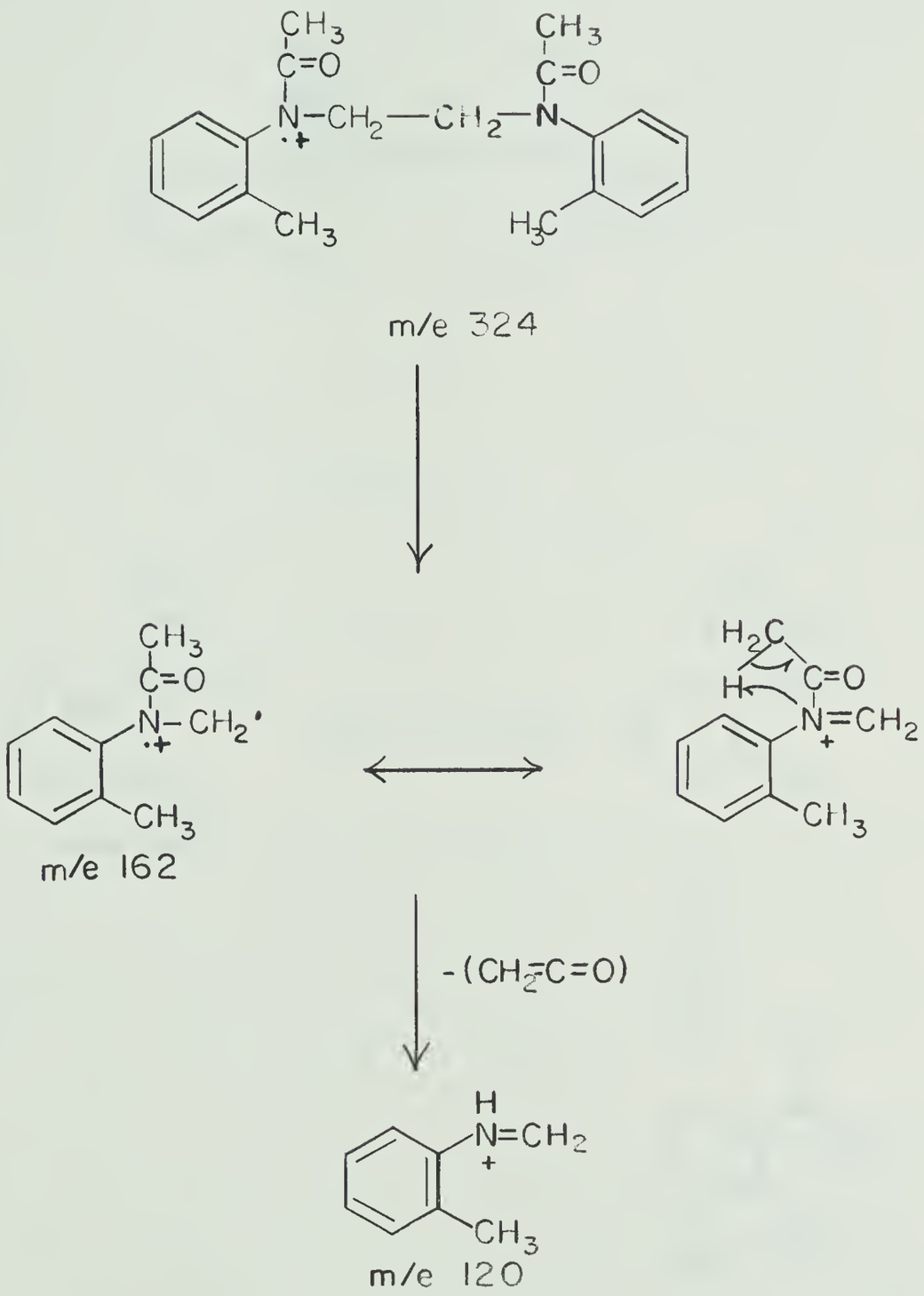
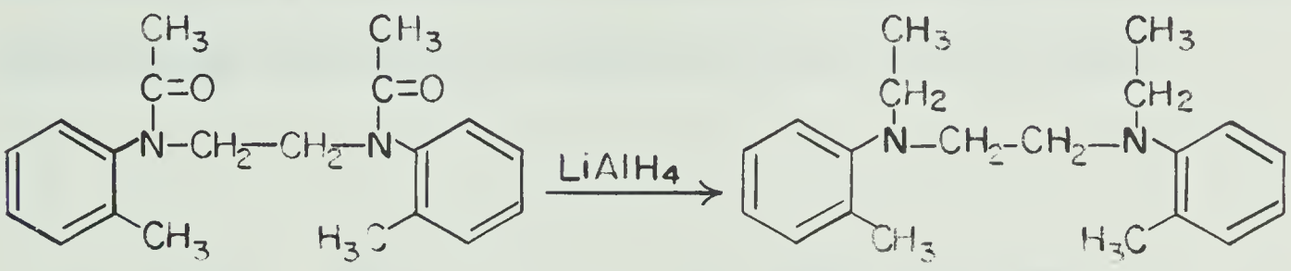


Figure 3 Path b

proposed, we felt further proof was required. A small quantity of the acetylated base was reduced with lithium aluminum hydride to give a tertiary amine, 8. That the reduction product was a pure compound was suggested by TLC (alumina, chloroform) and the tertiary amine was shown to be less polar than the amide. The molecular formula, $C_{20}H_{28}N_2$ (m/e 296), was established by high resolution mass spectrometry. Other fragment ions occur at m/e 148, 120, 91.



7 8

The mass spectral fragmentation pattern is consistent with that expected for the reaction product (see fig 4). β-Fission may give the radical at m/e 148. A rearrangement involving hydrogen transfer and the loss of ethylene may give the radical at m/e 120.

The tertiary amine has a UV spectrum (λ_{max}^{MeOH} 250 mμ and $\lambda_{max}^{H^+}$ 262, 270 mμ) which is nearly superimposable with that of a model compound, N,N-dimethyl-o-toluidine (λ_{max}^{MeOH} 243 mμ, λ_{max}^{MeOH} 260, 268 mμ).

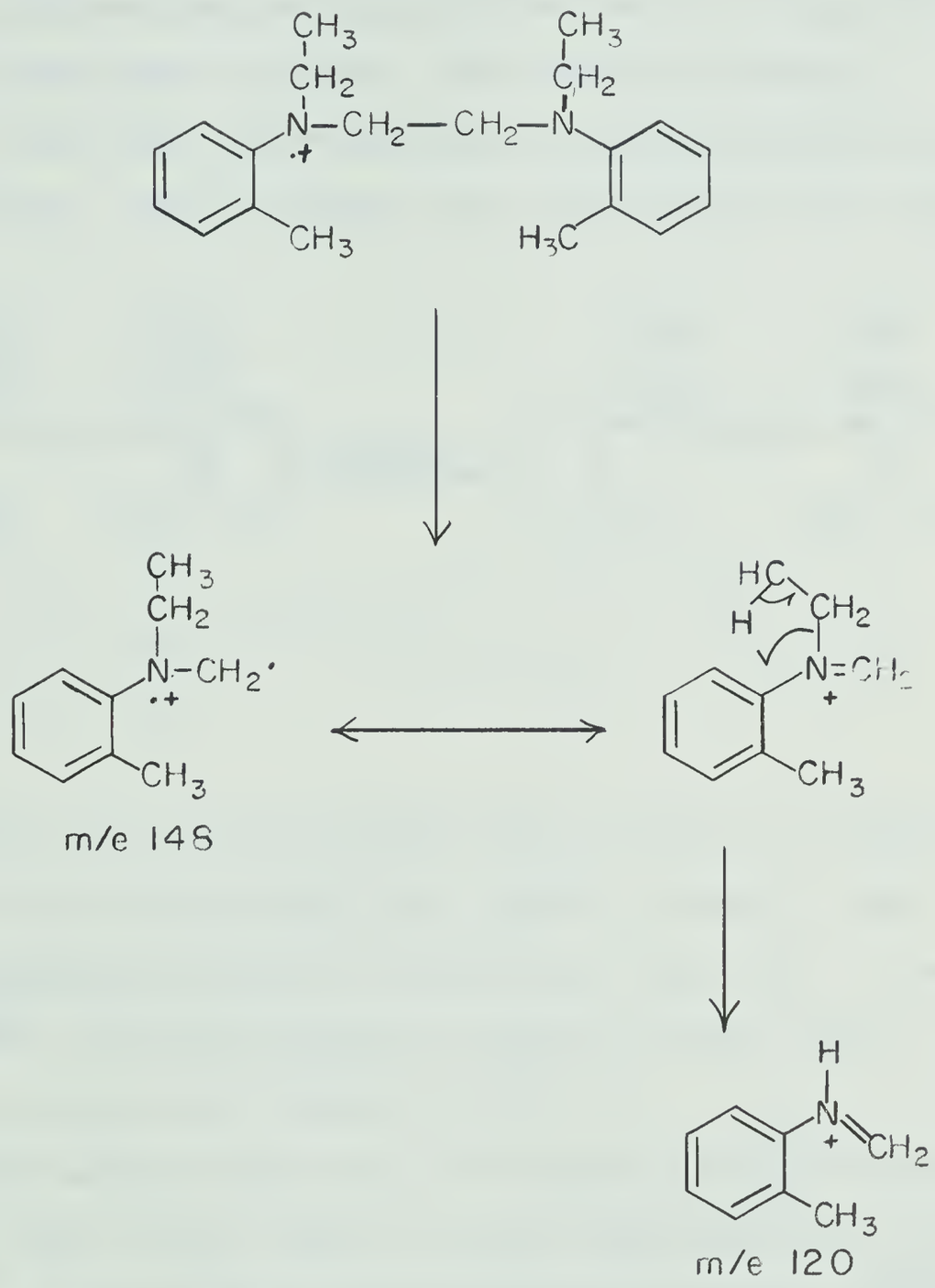
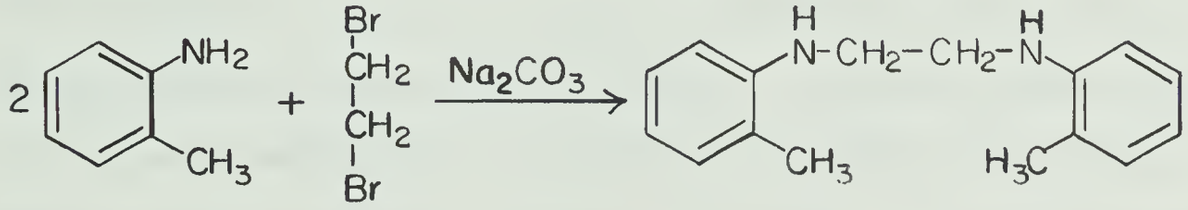
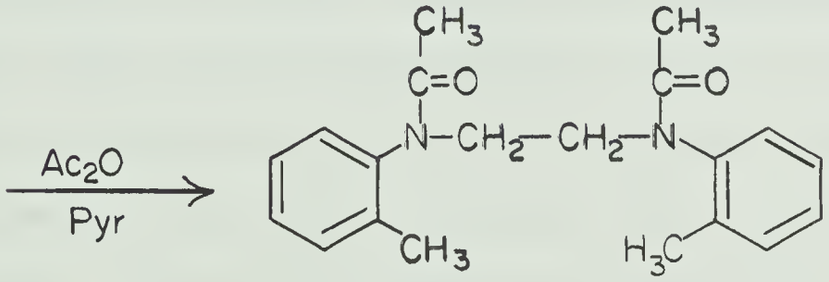


Figure 4

Because the reduction of the amide to the tertiary amine gave evidence for the structure proposed for the naturally occurring amide, we decided to synthesize the compound as final structure proof. o-Toluidine (2 parts) was heated with ethylene dibromide (1 part) in the presence of sodium carbonate for 1/2 hour. Unreacted o-toluidine was removed by steam distillation, the residue separated from water and crystallized from hot methanol.[38] The product was acetylated with acetic anhydride in pyridine, the crude amide being purified by crystallization from acetone.





The melting point of N,N´-diacetyl-N,N´-di-o-tolyl-1,2-

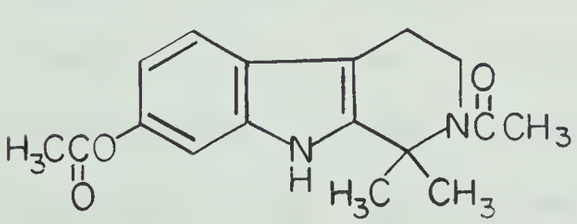
diaminoethane and the naturally occurring amide is 148-150° C. In addition their TLC behavior, IR and NMR spectra are identical.

The structure of the "naturally occurring" acetylated base was established unambiguously as N,N´-di-o-tolyl-1,2-diaminoethane. We felt an alkaloid of such a structure type was "unusual" and a literature survey was undertaken. We found that this type of compound is used as an antioxidant in silicone rubbers,[39] and we became suspicious that this compound may be an antioxidant present in silicone grease, which had been used only in the Soxhlet extraction with methanol of the ground, dried plant material. We therefore decided to see if we could isolate N,N´-di-o-tolyl-1,2-diaminoethane from silicone grease. Silicone grease was refluxed with methanol, the methanol decanted from excess grease and evaporated, leaving a residue. The residue was acetylated with acetic anhydride in pyridine. The mixed TLC of the crude acetylated material and N,N´-diacetyl-N,N´-di-o-tolyl-1,2-diaminoethane showed non-polar material but also a compound with R_f value identical with that of the synthetic material. We did not isolate N,N -diacetyl-N,N´-di-o-tolyl-1,2-diaminoethane *per se* from silicone grease but we feel that it is the probable source of this compound.

The second acetylated base isolated from *Shepherdia canadensis* was eluted from deactivated alumina with benzene -dichloromethane (1:1). The TLC behavior (alumina, chloro-

form) of this alkaloid suggested that it was a pure compound. It crystallizes from methanol (mp 212 - 214°C) and has an indole chromophore (λ_{max}^{EtOH} 227 mμ (ε=10,300), 291 mμ (ε=2,000)) in the UV. This is further supported by IR absorption bands at 3455 and 3300 cm^{-1}, indolic N-H, free and associated. The presence of an ester and an amide are indicated by carbonyl absorptions at 1750 and 1640 cm^{-1}, respectively. The NMR spectrum shows a one proton broad singlet at τ1.58, an ABX system of aromatic protons, an AA´BB´ set of triplets at τ6.36 and τ7.22, two three proton singlets at τ7.68 and τ7.75 due to the acetyl of an amide and an ester respectively, and a six proton singlet at τ8.16. The molecular formula, $C_{17}H_{20}N_2O_3$ (m/e 300), was established by high resolution mass spectrometry. Important fragment ions occur in the mass spectrum at m/e 285, 258, 243 (base), 201, 200.

The evidence presented thus far was consistent with either structure 9 or structure 10.



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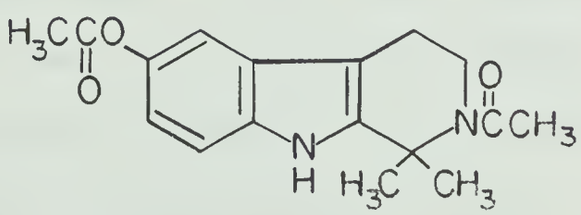

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Both would show a indolic N-H in the NMR; an ABX system characteristic of a 1,2,4-trisubstituted benzene ring; an AA´BB´ set of methylene protons; and two acetyl groups. The gem-dimethyl group could account for the six proton singlet at τ8.16, although this is at rather low field for such a grouping.

Examination of the mass spectral fragmentation pattern of the alkaloid isolated gives additional evidence for either structure 9 or 10 (see fig 5). The lack of intense peaks in the mass spectrum at low mass number is consistent with an aromatic and aliphatic molecule. The intense ion at m/e 285 corresponds to loss of a methyl radical, consistent with the presence of a methyl group on a carbon α to a nitrogen. Hydrogen transfer from the acetyl methyl to the nitrogen concurrent with loss of ketene may account for the base ion, m/e 243. Further loss of ketene by hydrogen transfer gives the ion at m/e 201, while loss of an acetyl radical gives the ion at m/e 200 and gives evidence for an acetoxy group.

Since the signal in the NMR assigned to the gem-dimethyl group occurred at lower field (τ8.16) than expected, a model system was prepared to see if N-acetylation markedly deshields the gem-dimethyl group, causing it to resonate at lower field. p-Toluenesulfonic acid catalyzed condensation of tryptamine with acetone in refluxing benzene yielded a Schiff base. The Schiff base was reacted with

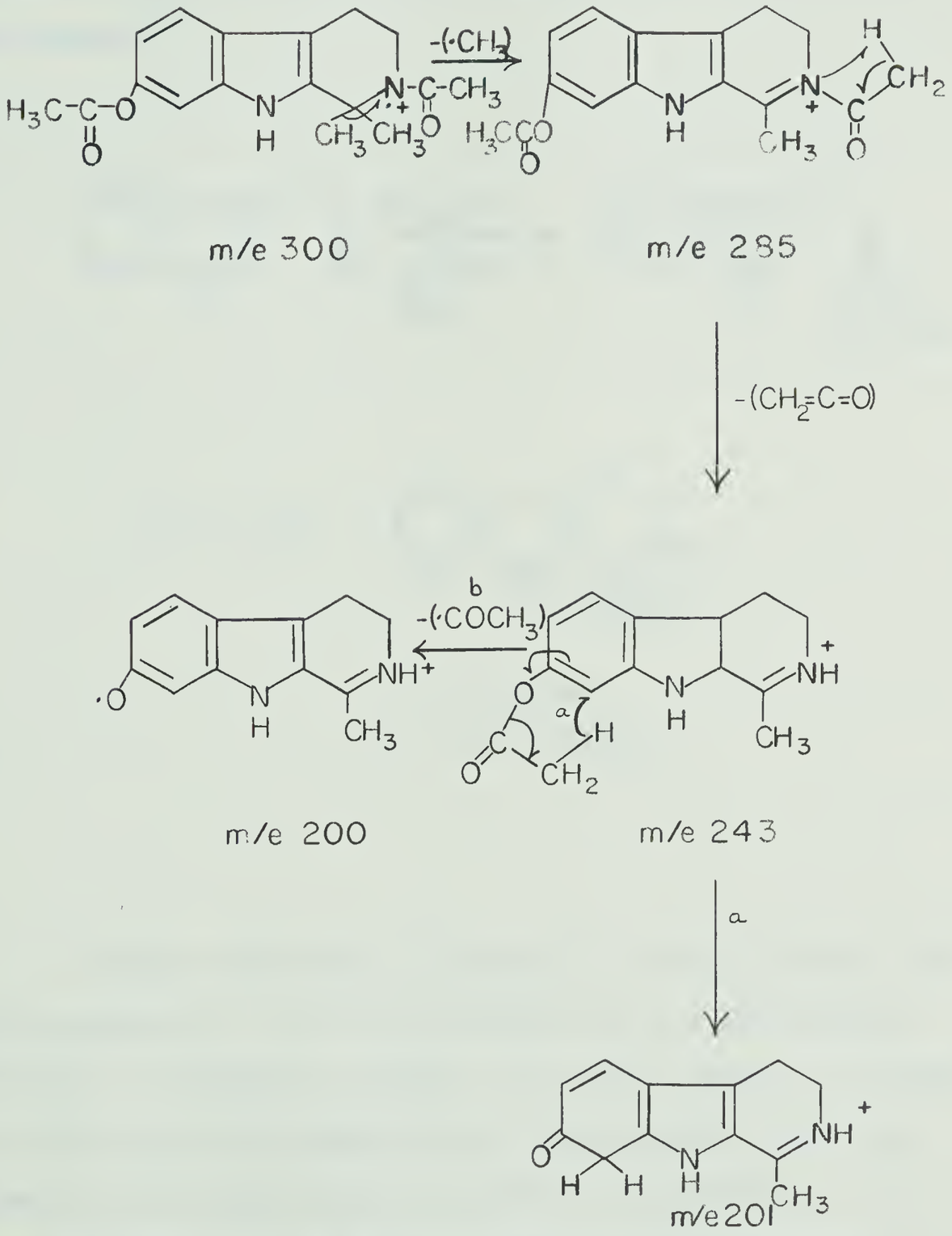
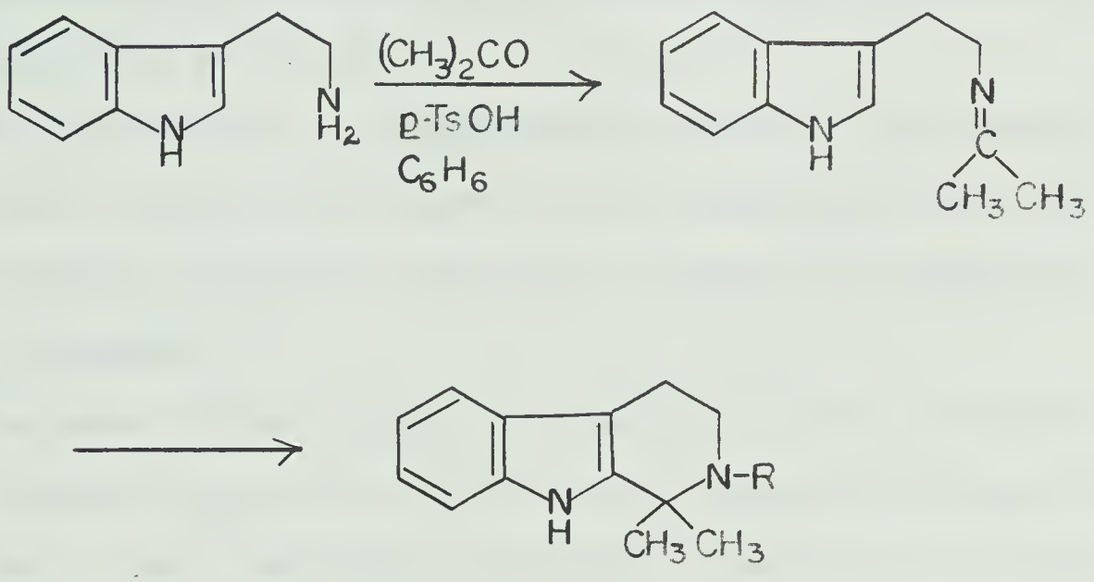


Figure 5

phosphorus oxychloride in reluxing benzene, then warmed with water to convert the phosphoramidic dichloride formed directly to the amine, 11. Compound 11 was purified by elution chromatography on deactivated alumina, and crystallized from benzene.[40]



11 R=H
12 R=COCH$_3$

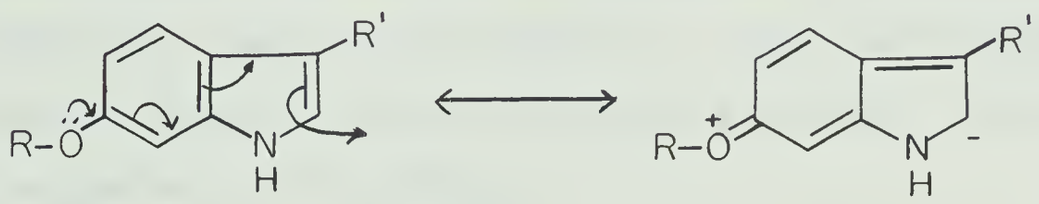
The NMR spectrum of compound 11 shows a broad, low-field singlet at τ2.20 of an indolic N-H; a four proton multiplet of aromatic protons; an AA´BB´ system of triplets at τ6.80 and τ7.30 indicative of the grouping -CH_2-CH_2-; a one proton, broad singlet at τ8.00 of an N-H and a six proton singlet at τ8.54 due to the gem dimethyl group.

Acetylation of compound 11 gave the N-acetyl (12), which crystallizes from acetone-water. The NMR spectrum of compound 12 has a three proton singlet at τ7.70 due to the methyl of the amide and a six proton singlet at τ8.09 showing that N-acetylation does cause considerable deshielding of the gem-dimethyl group. The methylene protons are also deshielded by N-acetylation, as the AA´BB´ system now occurs at τ6.30 and τ7.16.

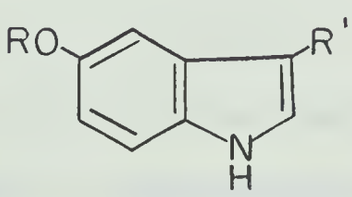
The presence of a gem-dimethyl group in the alkaloid isolated was further indicated by the experiments with the model compound and gives additional evidence for structures 9 and 10 proposed.

Because of the oxygenation pattern of the alkaloids in the Harmala group, we felt that the alkaloid isolated has structure 9. We considered a probable biosynthetic pathway for such an alkaloid. The fact that indole alkaloids are derived in part from tryptophan is generally accepted.[41] Recently, O'Donovan and Kenneally have shown that elaeagnine (tetrahydroharmine) is derived from tryptophan and acetaldehyde.[42] Hypothetically, then, an alkaloid of structure 9 could be derived from a suitably oxygenated tryptophan and acetone, or by methylation of tetrahydroharmine or a suitable precursor. Acétone or its biological equivalent is not a common biosynthetic building block, and we began to wonder if the alkaloid isolated was an alkaloid per se or an artifact. A literature search showed that tryptamines with

a C-6 oxygen substituent on the indole nucleus condense readily with acetone under mild conditions to form the ring closed condensation product, whereas unsubstituted tryptamines or tryptamines with a C-5 oxygen function do not.[40] A possible explanation is that oxygen substituents at C-6 enhance the nucleophilicity of the α-position of the indole nucleus, since one canonical form may be drawn which shows increased electron density at this position.



On the other hand, an oxygen substituent at C-5 cannot give such a canonical structure.



To determine if the alkaloid isolated was an artifact

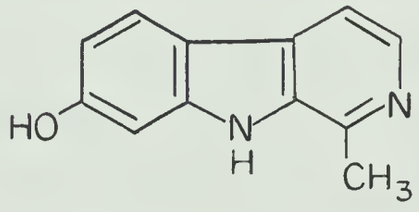
formed during extraction, the ground dried plant was extracted with reagent methanol since the technical methanol used previously contains acetone. As before, excess solvent was removed and the acidic and neutral material removed by acid-base extraction. The crude bases thus isolated were acetylated in the usual manner. The mass spectrum of the crude acetylated bases from the extraction with technical methanol showed a prominent ion at m/e 300, whereas the mass spectrum of the crude acetylated bases from extraction with reagent methanol did not. Elution chromatography of the latter did not yield any trace of the alkaloid. Thus the compound must be an artifact of the extraction procedure. Structure 9 is suggested on this basis.

The next alkaloid isolated from S. canadensis, was eluted from deactivated alumina with benzene-chloroform (4:1). That this alkaloid was a single compound was indicated by TLC (alumina, chloroform, methanol, 200:1; silica gel, chloroform-methanol, 20:1). It crystallizes readily from ethyl acetate and has a UV spectrum and IR spectrum similar to that of N,O-diacetyltetrahydroharmol, the IR spectrum differing only in the 1000 - 900 cm^{-1} region. The NMR spectrum of the crystalline base showed it to be a mixture of two alkaloids in approximately a 1:1 ratio. Attempts to separate the two alkaloids by chromatography were unsuccessful. However, separation was obtained by fractional crystallization from methanol. One of the alkaloids had

spectra identical with that of N,O-di-acetyltetrahydroharmol previously isolated from S. argentea, while the spectra of the other alkaloid was very similar to it.

To further substantiate the assignment of the major alkaloid isolated from S. argentea as N,O-diacetyltetrahydroharmol, and to correlate it with one of the alkaloids isolated from S. canadensis, we decided to synthesize N,O-diacetyltetrahydroharmol.

Harmol (13) was reduced with sodium in anhydrous ethanol, as described by T. Platanova and co-workers.[35]



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The crude residue was acetylated in the usual manner and purified by elution chromatography on deactivated alumina. N,O-diacetyltetrahydroharmol crystallizes from ethyl acetate (mp 202°C). The melting point of the alkaloid assigned structure 1, as well as the mixed melting point of the naturally occurring alkaloid and N,O-diacetyltetrahydroharmol is 202°C. Their spectra are identical in all respects.

The other alkaloid, given the trivial name N,O-diacetylshepherdine by this laboratory, was separated from N,O-

diacetyltetrahydroharmol by fractional crystallization from methanol. It has a melting point of 192 - 194°C, and a mixed melting point with N,O-diacetyltetrahydroharmol of 165 - 175°C. An indole chromophore is indicated by the UV spectrum (λ_{max}^{EtOH} 225 mμ (ε=31,400), 281 mμ, (ε=6,400)), and verified in the IR spectrum (3450, 3275 cm^{-1}). Carbonyl absorptions of an ester (1745 cm^{-1}) and an amide (1630 cm^{-1}) are also present, the rest of the IR differing from that of N,O-diacetyltetrahydroharmol only in the fingerprint region (1000 - 900 cm^{-1}). The NMR spectrum shows a one proton, broad singlet at τ1.14; an ABX system of aromatic protons; a one proton quartet at τ4.30, J = 6 cps and a three proton doublet at τ8.62, J = 6 cps indicating a $-CHCH_3$ group; an ABCD system of methylene protons at τ6.07 (1H), τ6.64 (1H), τ7.34 (2H), due to the equatorial proton on the carbon α-to the amide nitrogen, the axial proton on the carbon α-to the amide nitrogen, and the methylene protons on the carbon β-to the amide nitrogen, respectively; and finally two three proton singlets of an amide (τ7.71) and ester (τ7.80). The molecular formula, $C_{16}H_{18}N_2O_3$ (m/e 286), was established by high resolution mass spectrometry. Other intense fragment ions occur at m/e 271, 229, 201, 187.

The spectral evidence presented to this point is consistent with either structure 1 or structure 2. Again both would display low field indole N-H, and ABX system of aromatic protons characteristic of a 1,2,4- trisubstituted

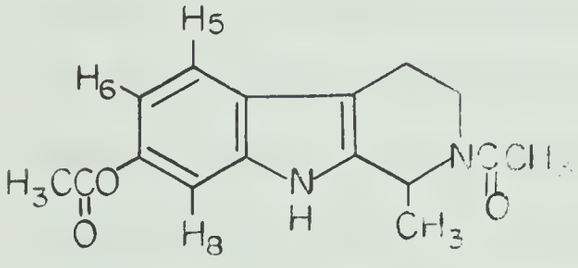
benzene ring, a lowfield methine coupled to a $-C-CH_3$, an ABCD system of methylene protons, and two acetyl groups.

The mass spectral fragmentation pattern is consistent with either of the structures proposed, and is the same as that of N,O-diacetyltetrahydroharmol previously discussed.

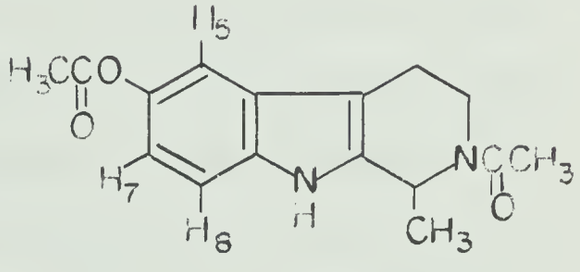
We favored the assignment of structure 2 for N,O-diacetylshepherdine on the basis of the following points:

1) the infrared spectrum was similar to N,O-diacetyltetrahydroharmol except in the 1000 - 900 cm^{-1} region.

2) in the NMR spectrum, the chemical shifts of the ABX system of aromatic protons of N,O-diacetyltetrahydroharmol and N,O-diacetylshepherdine were similar but not identical.



1

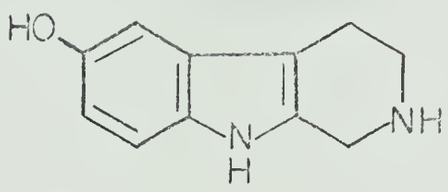


2

H-5 2.99 J=8 cps	H-5 2.92 J=1 cps
H-6 3.24 J=2,8 cps	H-7 3.22 J=1,8 cps
H-8 2.69 J=2 cps	H-8 2.80 J=8 cps

3) the mixed melting point of N,O-diacetylshepherdine with N,O-diacetlytetrahydroharmol was depressed from that of pure N,O-diacetyltetrahydroharmol.

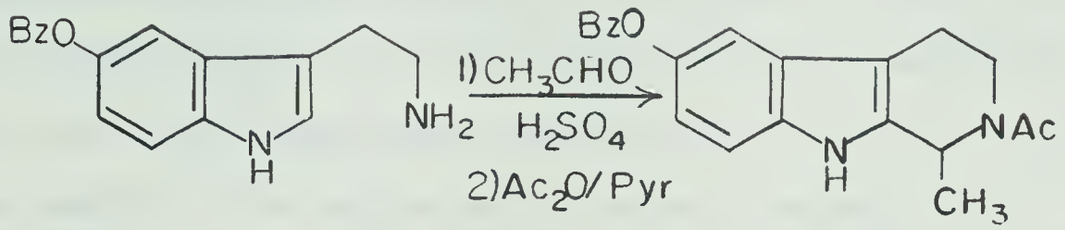
4) recently, Taylor and co-workers[43] have characterized a new harmala-type alkaloid, plectocomine (14), which has an oxygen function at C-6.



14

5) and lastly, serotonin was isolated (see below) from S. canadensis.

We decided to synthesize compound 2 to further substantiate the assignment. Sulfuric acid catalyzed condensation of 5-benzyloxytryptamine with acetaldehyde yielded a crystalline precipitate (15) which was acetylated in the usual manner and purified by elution chromatography on deactivated alumina.[44] Compound 15 crystallizes from acetone-methanol. Catalytic hydrogenolysis (Pd-C) of compound 15, followed by acetylation gave compound 2. Compound 2 crystallizes from methanol. The mp of the naturally occurring alkaloid, N,O-diacetylshepherdine, is 192 - 194°C, the mixed mp with synthetic compound 2 is 192 - 194°C. Their IR are superimposable.



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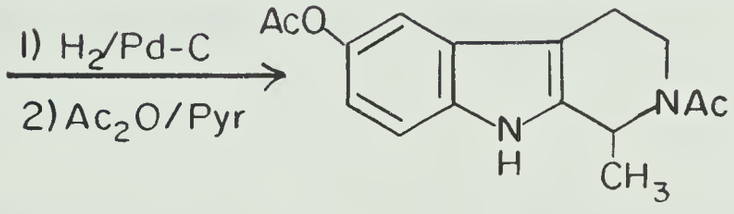


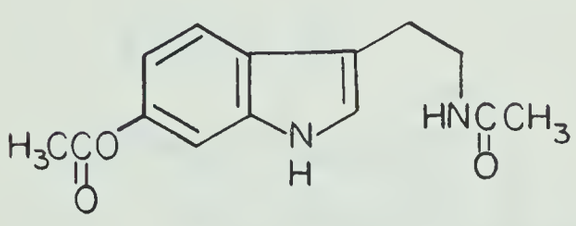
2

The last alkaloid characterized from S. canadensis was isolated by elution chromatography on deactivated alumina with chloroform. The TLC (alumina, chloroform-methanol, 50:1) suggested it was a pure compound but attempted crystallization was unsuccessful. The presence of an indole chromophore is indicated by the UV spectrum (λ_{max}^{EtOH} 224 mμ (ε=27,000), 287 mμ (ε=5,500)) and supported by absorption bands in the IR at 3500 and 3325 cm^{-1}. As well, carbonyl absorptions of an ester (1750 cm^{-1}) and an amide (1675 cm^{-1}) are present. The NMR spectrum shows a

broad singlet at τ-0.20 due to indolic N-H, an ABX system of aromatic protons, a one proton singlet at τ2.86, an AA´ BB´system of methylene protons at τ6.58 and τ7.15, a one proton broad singlet at τ6.77 due to N-H of a secondary amide, and two three proton singlets at τ7.79 and τ8.15 due to the acetyl of an amide and an ester, respectively.

The molecular formula, $C_{14}H_{16}N_2O_3$ (m/e 260), was established by high resolution mass spectrometry. Other intense fragment ions occur at m/e 201, 188, 159, 146.

To this point, the evidence presented is consistent with either structure 16 or structure 17.



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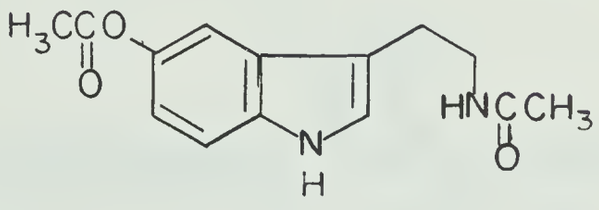


17

Both would show a low field indolic N-H, an ABX system of aromatic protons, a one proton singlet in the aromatic region due to the proton on the carbon α-to the indolic N-H, an AA´BB´ system of methylene protons, a singlet due to the N-H of an amide and two acetyl groups.

Spin-spin decoupling experiments allow the assignment of the aromatic protons. There is a one proton doublet which is ortho coupled at τ2.68 J=8 cps, a one proton

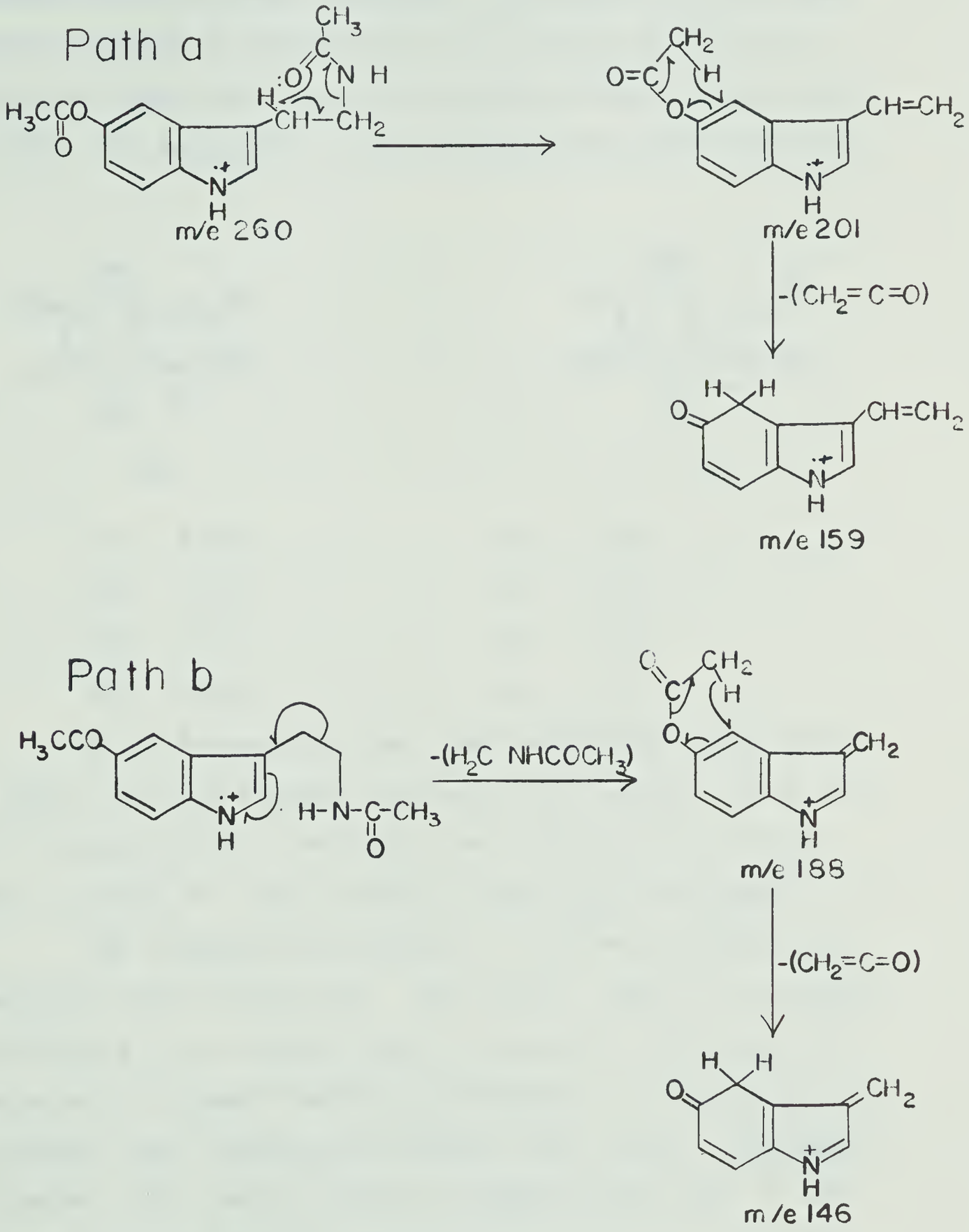
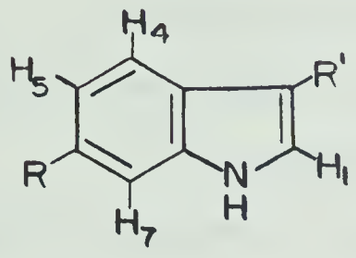
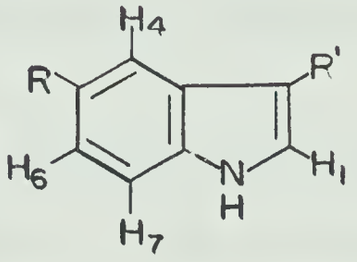

Path a
m/e 260
m/e 201
-(CH2=C=O)
m/e 159
Path b
-(H2C NHCOCH3)
m/e 188
-(CH2=C=O)
m/e 146

Figure 6

doublet which is meta coupled at τ2.75, J=2 cps and a one proton doublet of doublets at τ3.21, J=2,8 cps which is ortho and meta coupled. The isolated aromatic proton is at τ2.86. The assignment is consistent with either structure.



16



17

H-1	2.86	H-1	2.86
H-4	2.68	H-4	2.75
H-5	3.21	H-6	3.21
H-7	2.75	H-7	2.68

It is interesting that these experiments also showed a small coupling between the indolic N-H and H-4. This type of coupling has not previously been reported for indoles but is known for fused aromatic rings e.g., quinoline.[45]

The fragmentation pattern of the mass spectrum also supports either structure (see fig 6). Again, a molecule consisting of an aromatic and an aliphatic part would be expected to fragment mainly by breaking of the bonds in the aliphatic part leaving the aromatic part intact. Hydrogen transfer with loss of a glycyl fragment gives rise to the ion at m/e 201, while further hydrogen transfer with loss of ketene gives rise to the base peak m/e 159. An alternate

fragmentation pathway may result from fission of the most activated bond (α-to the indole nucleus and β-to the amino group) to give the fragment ion m/e 188. Further hydrogen transfer accompanied by loss of ketene may give rise to the intense ion m/e 146 (87%).

We favored the assignment of the acetoxy function to C-5(17) because of the following:

1) the acetylated alkaloid was isolated from the plant extraction with technical methanol. If the acetoxy function was at C-6, the condensation product should have formed.

2) the fingerprint region (1000 - 900 cm^{-1}) of the IR spectrum corresponds more closely to compound 2 than to N,O-diacetyltetrahydroharmol (1).

3) the chemical shifts and coupling constants of tryptophan hydrochloride as reported by Kirby and Shah correspond closely to those of the alkaloid isolated.[47]

Because we felt further structure proof was necessary, the synthesis of compound 17 was undertaken. Acetylation of an authentic sample of serotonin creatinine sulfate (Aldrich Chemical Co), followed by removal of creatinine by salting out with acetone and chromatography of the crude residue on deactivated alumina led to the isolation of N,O-diacetylserotonin. The IR spectrum of N,O-diacetylserotonin the naturally occurring alkaloid are superimposable.

THE NEUTRAL COMPONENTS OF CLEMATIS LIGUSTICIFOLIA

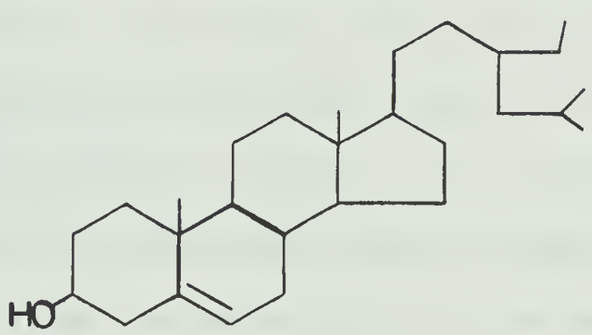
Ground dried roots (260 g) were extracted with 5% aqueous methanol in a Soxhlet extractor. A flocculent precipitate was filtered from the aqueous methanol, and excess solvent removed. The tar-like residue was dissolved in acetone. Initially, separation into acetone soluble and acetone insoluble fractions was attempted with a homogenizer but this method was discarded in favor of Soxhlet extraction with acetone. Excess acetone was removed and the residue (37.2 g) dissolved in chloroform-t-butyl alcohol (20:1). The basic and acidic material was removed by extraction with 5% aqueous hydrochloric acid and 5% aqueous potassium hydroxide respectively, leaving the neutral material in the chloroform layer. The aqueous acidic fraction was basified and continuously extracted with chloroform yielding 0.1 g crude bases (0.004%). Chromatography (column and thin layer) revealed that the basic fraction was composed of several components (>4). Attempts to isolate pure components were not successful. Because of the small amount of basic material present, the bases were not further investigated.

We then investigated the neutral fraction (6.5%) which was left in the chloroform layer after removal of acidic and basic material. Elution chromatography on alumina led to the isolation of two crystalline compounds in very small amounts. Elution chromatography with silicic

acid led to the isolation of the same crystalline compounds but in better yields.

The first crystalline compound isolated from the neutral fraction was thought to be a steroidal alcohol on the basis of its infrared and mass spectra. It crystallizes from hot methanol-acetone, mp 136 - 138°C. The IR spectrum shows an alcohol, 3615 and 1150 cm^{-1}. The mass spectrum shows a small molecular ion at m/e 414, and a M - 18 peak at m/e 396 which is characteristic of an alcohol. The remaining fragmentation pattern shows clusters of peaks, the corresponding peak in each cluster being 14 mass units apart.

A literature survey showed that β-sitosterol (19) has a melting point 136°C, which is close to that of the steroidal alcohol isolated.[48]



19

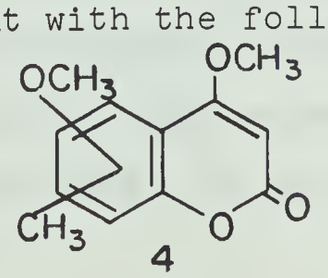
We thus decided to prepare a derivative of the naturally occurring alcohol. Acetylation with acetic anhydride in pyridine gave the O-acetyl derivative, the IR spectrum of which shows no O-H absorption but has a strong carbonyl absorption at 1735 cm^{-1}. The acetate was crystal-

lized twice from methanol and the mp (135 - 137°C) corresponded well with the mp reported for β-sitosterol acetate (134°C)[48]

An authentic sample of β-sitosterol was compared with the naturally occurring steroidal alcohol. Their mp (135 - 136°C), mixed mp (135 - 136°C), and IR spectral are identical in all respects.

The second crystalline compound isolated from C. ligusticifolia crystallizes from methanol. It has a mp 185 - 187°C and the UV spectrum is coumarin-like (λ_{max}^{EtOH} 223 mμ (ε=18,600), 308 mμ (ε=13,000). The IR spectrum shows an α,β-unsaturated carbonyl at 1700 cm^{-1} and 1605 cm^{-1}, and an aromatic ether at 1160 and 1050 cm^{-1}. The NMR spectrum shows an AB system of aromatic protons at τ3.36, J=2.5 cps and 3.42, J=2.5 cps; a one proton singlet at τ4.49 suggesting a vinyl proton, two three proton singlets at τ6.08 and τ6.18 characteristic of methoxyl groups and a three proton singlet at τ7.40 due to a methyl. The molecular formula, $C_{12}H_{12}O_4$ (m/e 220), was established by high resolution mass spectrometry. Other intense ions in the mass spectrum occur at m/e 192, 177, 149.

The spectral information presented to this point is consistent with the following structures.



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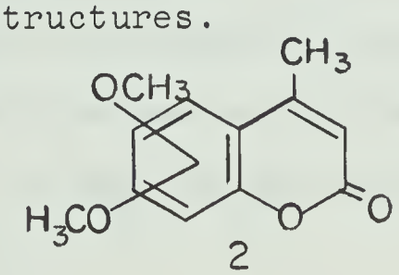
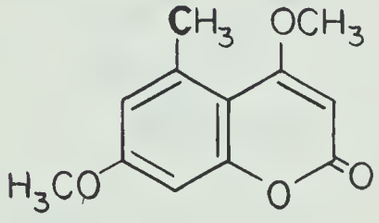


2

All would show in the NMR an AB system of aromatic protons meta to one another as suggested by the coupling constant J=2.5 cps; a low field vinylic proton; two methoxyl groups; and a low-field methyl.

The mass spectral fragmentation pattern of the naturally occurring coumarin is also consistent with the structures proposed.[49] (see fig 7) Initial loss of carbon monoxide from the molecular ion (m/e 220) gives the radical ion at m/e 192. Loss of a methyl radical (m/e 177) followed by further loss of carbon monoxide may account for the intense peak at m/e 149.

Of the six possible structure combinations proposed, structure 20 and structure 21 were considered most probable from a biosynthetic point of view.



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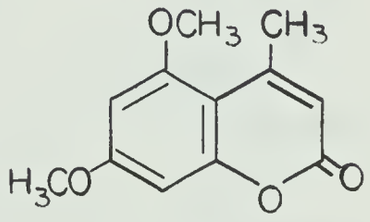
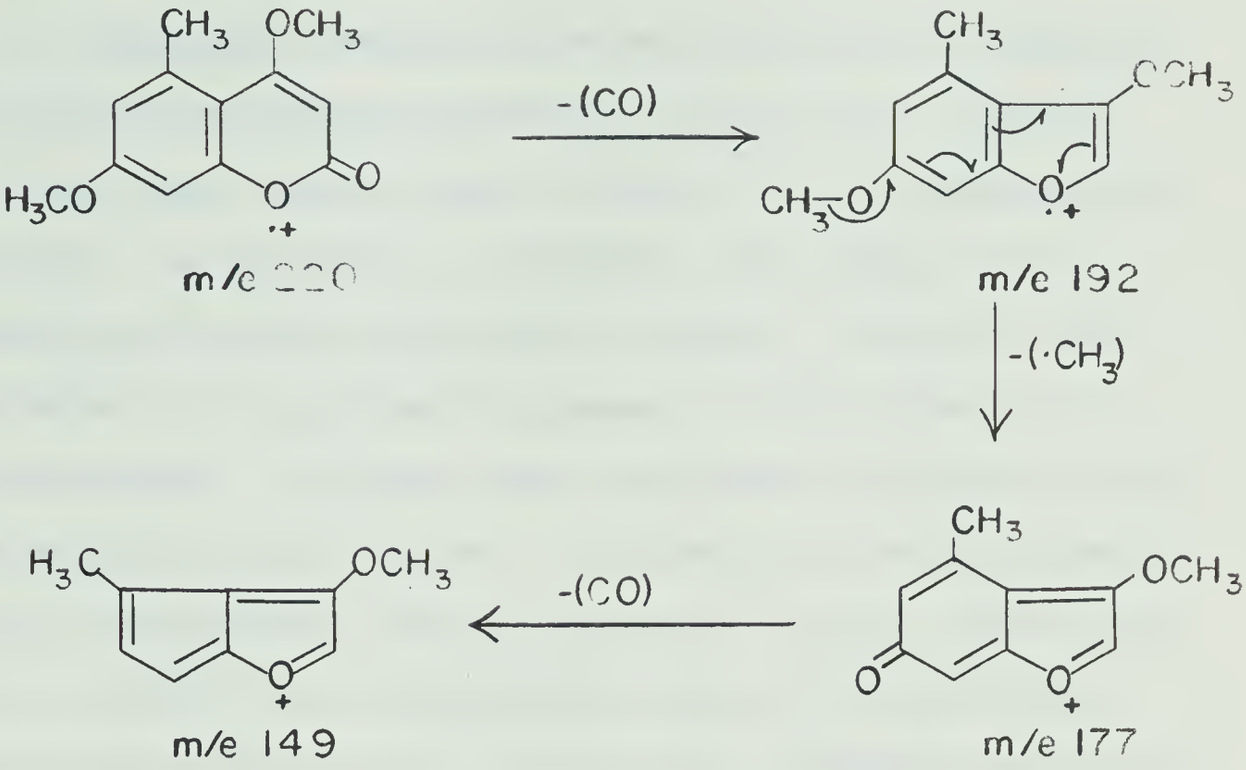


21

Evidence from feeding experiments indicate that shikimic acid derived phenylpropanes are the biogenetic precursors of coumarins,[50] and coumarins with oxygen substituents in the C-7 and C-6, C-7 and C-5, C-7 and C-4 positions occur in

For example



Or

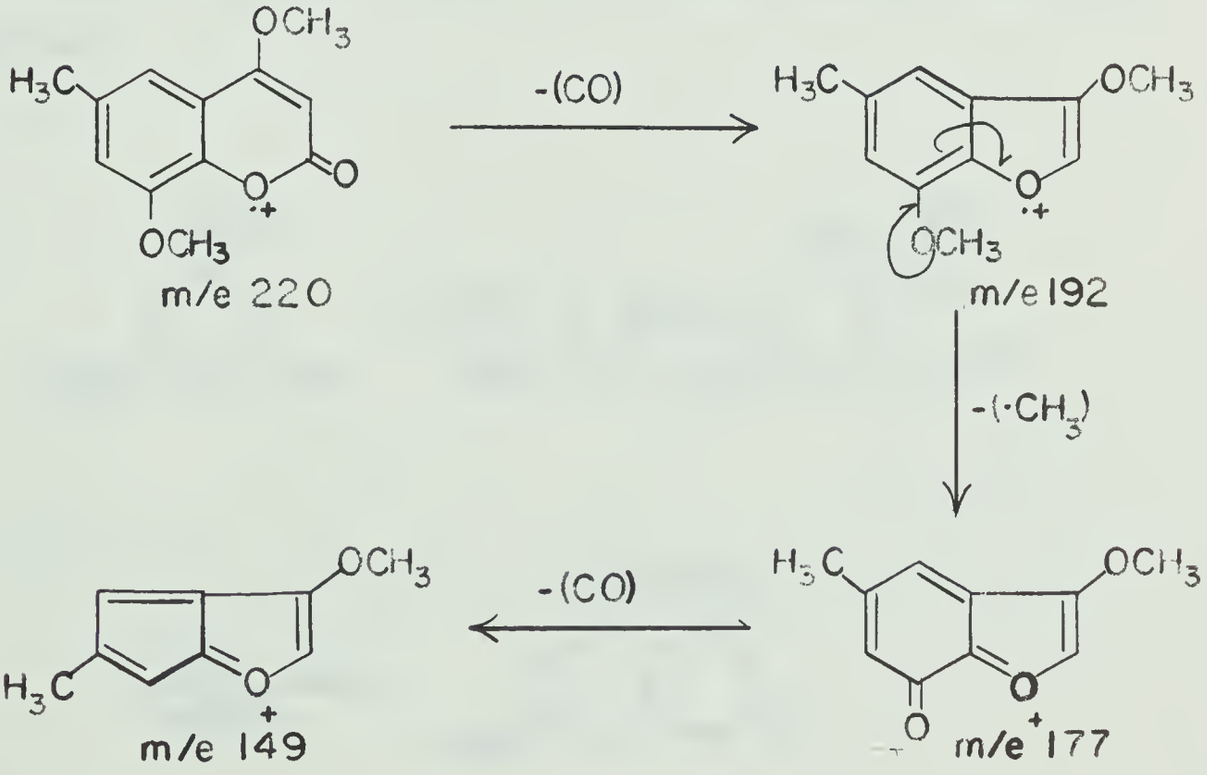
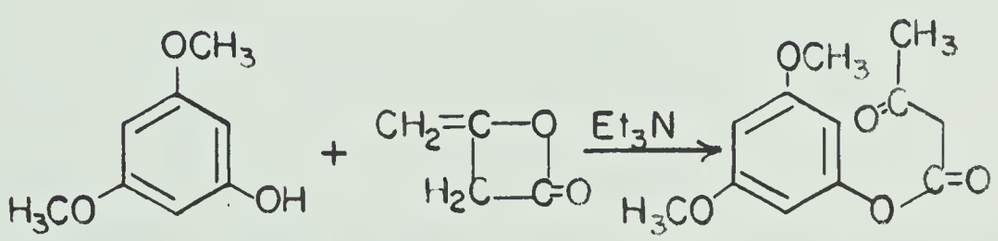
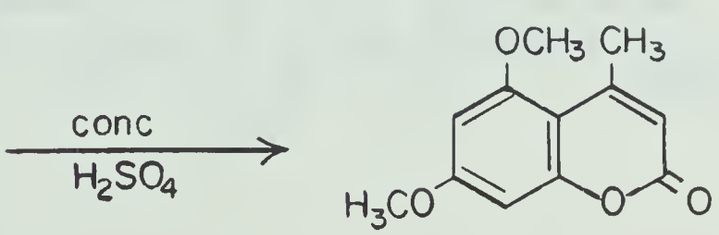


Figure 7

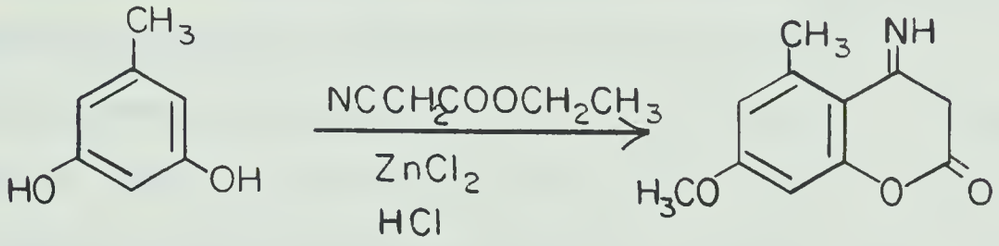
nature. ie, naturally occurring coumarins almost invariably are oxygenated at C-7.

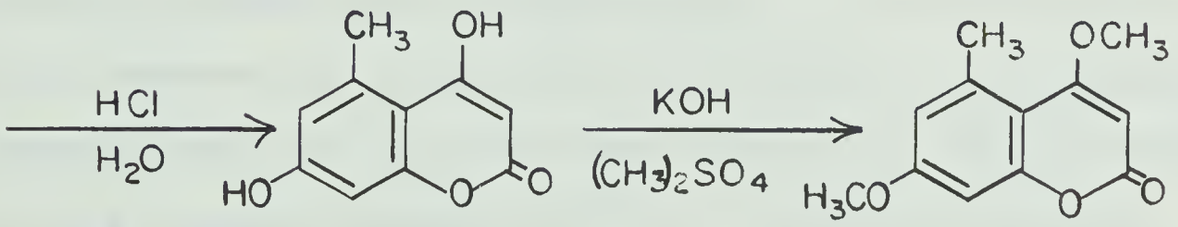
Gupta and Seshadri have reported the synthesis of 5,7-dimethoxy-4-methylcoumarin (21), mp 179 - 180°C,[51] although this coumarin has not been isolated from natural sources. We decided to synthesize this coumarin (21), using the procedure described by Lacey.[52] Diketene was condensed with 3,5-dimethoxyphenol in the presence of triethylamine. Sulfuric acid catalyzed cyclization of the condensation product gave 5,7-dimethoxy-4-methylcoumarin which crystallizes from hot methanol, mp 173 - 175°C, rep 179 - 180°C.[52] The mixed melting point of the naturally occurring coumarin and 5,7-dimethoxy-4-methylcoumarin was 148 - 150°C. As well, their UV and NMR spectra were non-identical.





Since we had shown by synthesis that structure 21 was not the structure of the coumarin isolated from C. ligusticifolia, we undertook the synthesis of 4,7-dimethoxy-5-methylcoumarin, (20). Zinc chloride-hydrochloric acid catalyzed condensation of orcinol with ethylcyanoacetate, followed by acid catalyzed hydrolysis gave 4,7-hydroxy-5-methylcoumarin, (21) mp 282 - 284°C, rep. 266 - 267°C.[53] The product was methylated with an alkaline solution of dimethylsulfate, then purified by chromatography on silicic acid.





22

The coumarin isolated crystallized from hot methanol, mp 189 - 190°C. Its IR and NMR spectra were identical with that of the naturally occurring coumarin, however the UV spectra differed slightly. Both the naturally occurring coumarin, and the synthetic coumarin were recrystallized from methanol. Their mp, mixed mp is 190 - 191.5°C and their UV spectra are identical. Thus the coumarin isolated from C. ligusticifolia was shown to be 4,7-dimethoxy-5-methylcoumarin by synthesis.

After this work was completed, two spectrometric methods were reported which would have allowed us to distinguish between structure 20 and structure 21. Grigg and co-workers[54] have reported an NMR solvent shifts technique which enables the position of methyl, methoxyl and aromatic protons in coumarins to be determined. On the other hand, Johnstone and co-workers,[55] have used mass spectrometric methods. They have shown that coumarins with a 4-methyl substituent have a modified fragmentation pattern.

To the best of our knowledge this is the first natural coumarin with a methyl group in the C-5 position. It is interesting to note that cyclization of a polyacetate precursor such as "X" would lead directly to the skeleton of this coumarin together with the oxygenation pattern.

mass spectrum: m/e 242.1617 calcd for $C_{17}H_{22}O$, 242.1678 meas (21), 173(100), 146(12), 131(12), 123(9), 97(84), 91(43), 69(10), 55(9), 43(12), 41(28).

3-Benzylidene-1,4,4-trimethylbicyclo[3.2.0]heptane, 17

Benzylidene ketone 15 (0.21 g), 85% hydrazine hydrate (29 ml), hydrazine dihydrochloride (0.73 g) and triethylene glycol (174 ml) were heated at 120° for two hours. Potassium hydroxide pellets (1.1 g) were added and the temperature was slowly raised to 230°, allowing the low boiling material to distil. The reaction mixture was held at that temperature for 1.5 h then cooled, diluted with water and extracted with petroleum ether. The pet ether fraction was washed with water, dried, concentrated and the residual oil substilled, bp 100° (0.5 mm). Yield 0.11 g (58%).

Anal. Calcd for $C_{17}H_{22}$: C, 90.20; H, 9.80. Found: C, 89.87; H, 9.77.

uv_{max}^{MeOH}: 249 nm (ε = 2,150).

$ir_{(film)}$: 1610 (conj C=C), 1500 cm^{-1}.

$nmr_{(CDCl_3)}$: τ cr at 2.83 (m, 6, ArH and ArCH=C), 8.52 (s, 2, CH_2C=C), 8.73 (s, 6, CH_3), 8.95 (s, 3, CH_3).

mass spectrum: m/e 226.1721 calcd for $C_{17}H_{22}$, 226.1715 meas (6), 198(9), 145(100), 129(11), 91(39).

Attempted oxidative cleavage of the benzylidene compound 17

Ozone was bubbled through a solution of compound

17 in the solvent shown until a blue color indicated the presence of excess ozone. The reaction mixture was allowed to stand at varying temperatures for varying lengths of time. Excess ozone was removed and the reaction mixture subjected to an oxidative workup. In most cases only unreacted benzylidene compound was recovered.

Conditions Used for Ozonolysis

Solvent	Time(h)	Temp	Result
ethyl acetate	1.5	-10	recovered benzylidene
methylene chloride	4.5	-70	recovered benzylidene
acetic acid	6	24	low recovery of a mixture

Ozone was bubbled slowly through a solution of benzylidene compound 17 (0.250 g) in acetic acid (10 ml) for six hours. Excess ozone was removed and the acid solution diluted with water, then neutralized ($NaHCO_3$). The aqueous solution was continuously extracted with ether. The ether fraction was dried and concentrated to a solid (0.082 g). The solid which was mainly one compound (tlc, silica gel, benzene) was recrystallized from ethyl acetate mp 138.5-140°.

Anal. Calcd for $C_{12}H_{18}O_2$: C, 74.19; H, 9.34. Found: C, 74.23; H, 9.49.

$ir_{(CHCl_3)}$: 3300-2500 (br OH), 1730 sh, 1695 (C=O) cm^{-1}.

$nmr_{(CDCl_3)}$: no protons below τ 2, no exchangeable protons,

be chromatographed were dissolved in a minimum amount of non-polar solvent and applied to the column. The column was then eluted with solvents in the order of increasing polarity.

EXTRACTION AND ISOLATION OF THE CRUDE BASES OF *SHEPHERDIA ARGENTEA*

The bark of the roots (880 g) of *Shepherdia argentea* were ground, air dried, and extracted with 5% aqueous methanol in a Soxhlet extractor. Excess methanol was removed in vacuo, water added, then traces of methanol removed in vacuo. Five percent aqueous hydrochloric acid was added until the pH was less than 5 and the insoluble material filtered and discarded. The acidic and neutral components were removed from the filtrate by ether extraction. The acidic solution was basified with aqueous ammonia to pH 10 and the resultant precipitate filtered. The basic filtrate was continuously extracted with ether for 48 hours. The ether extract was dried (anhydrous magnesium sulfate), and excess solvent removed *in vacuo* to give 1.7 g crude bases (0.2%). Further purification of the crude alkaloidal material was achieved by a second acid-base extraction.

ACETYLATION

Referred to throughout the text as "acetylation in

the usual manner"; the base(s) were dissolved in one part pyridine, two parts acetic anhydride added and the reaction mixture allowed to stand at room temperature at least 12 hours. Excess solvents were removed by azeotropic distillation with toluene *in vacuo*.

ISOLATION OF ACETYLATED BASES OF *SHEPHERDIA ARGENTEA*

Crude acetylated bases were separated by elution chromatography on Woelm alumina, activity 3. Two fractions were obtained:

1) Non-polar bases, eluted with benzene-chloroform 10:1. (36%)

2) N,O-diacetyltetrahydroharmol, eluted with benzene -chloroform, 5:1. (24%)

N,O-DIACETYLTETRAHYDROHARMOL(1)

Compound 1 was isolated by elution chromatography on deactivated alumina with benzene-chloroform (5:1). Compound 1 crystallizes from ethyl acetate, mp 202°C.

UV spectrum: λ_{max} 229 mμ (ε=21,000), 282 mμ (ε=3,500).

IR spectrum: ν_{max} 3450, 3300 cm^{-1} (indole N-H); 1745 cm^{-1} (C=O of an ester); 1625 cm^{-1} (C=O of an amide).

NMR spectrum: τ1.07, singlet (N-H); 2.72, doublet J=8 cps (H-5); 3.01, doublet J=2cps (H-8); 3.26, doublet of doublets J=2,8cps (H-6); 4.35, quartet J=7cps (H-1); 6.08, multiplet (H-3e); 6.65, multiplet (H-3a); 7.34, multiplet

(H-4e,4a); 7.73, singlet (N-CO-$C\underline{H}_3$); 7.82, singlet (O-CO-$C\underline{H}_3$); 8.66, doublet J=7cps (C_1-$C\underline{H}_3$). Assignments have been verified by spin-spin decoupling experiments.
Mass spectrum: m/e 286 ($C_{16}H_{18}N_2O_3$; found 286.1317, calculated 286.1317), 271, 244, 229 (base), 201, 187.
ORD spectrum: no rotation

TETRAHYDROHARMOL (3)

Compound 3 was isolated by elution chromatography of the crude bases of S. argentea, on deactivated alumina using chloroform-methanol (20:1). Compound 3 could not be crystallized, mp 254.5° C; reported 256° C.[55]
UV spectrum: λ_{max} 229 mμ(logε = 4.57), 270 mμ(logε = 3.77), 299 mμ(logε = 3.85).
IR spectrum: ν_{max}^{nujol} 3380, 3265, 3245 cm^{-1} (N-H stretch), 1620, 1560 cm^{-1}.
Mass spectrum: m/e 202 ($C_{12}H_{14}N_2O$; found 202.1104, calculated 202.1106), 187 (base), 172, 159.

Tetrahydroharmol was dissolved in a minimum amount of hot methanol and methanolic HCl added until the solution was acidic. Excess solvent was removed. Tetrahydroharmol hydrochloride crystallizes from methanol-ether, mp 230°C (dec) reported 235° C[35]. Several months later, the same sample of tetrahydroharmol hydrochloride melted at 235°C without decomposition.

ISOLATION OF NON-POLAR BASES OF *SHEPHERDIA ARGENTEA* BY GAS CHROMATOGRAPHY[36]

Gas chromatographic analysis was performed with an Aerograph Manual Temperature Programmed Gas Chromatograph Model A-90-P_3, equipped with a thermal conductivity detector.

Samples of non-polar acetylated bases (20µl) were separated on a 10' × 1/4", 5% SE-30 column, column temperature 205°C, flow rate 20 ml/min. The following, in order of increasing retention time were isolated. Mass spectra were taken on each fraction.

Number	Retention time	Molecular ion
5	6.5 min	m/e 113 ($C_6H_{11}NO$)
	8.8 min	m/e 157 ($C_8H_{15}NO_2$)
	9.5 min	----
	10.6 min	----
	16.0 min	m/e 163 ($C_{10}H_{13}NO$)
6	23.3 min	m/e 165 ($C_9H_{11}NO_2$)

N-ACETYLPYRROLIDINE (5)

Compound 5 was isolated by gas chromatography and had the following mass spectrum: m/e 113 ($C_6H_{11}NO$; found 113.0844, calculated 113.0841), 85, 71, 70, 68, 55, 44, 43.

Pyrrolidine was acetylated in the usual manner and purified by distillation at reduced pressure, bp_{10} 85° C;

reported bp_{15} 108°C.[56] The mass spectra of N-acetylpyrrolidine and compound 5 are identical.

N-ACETYL-p-ANISIDINE (6)

Compound 6 was isolated by gas chromatography. It was a crystalline compound, mp 123 - 124 C, reported 130°C[57] and had the following mass spectrum: m/e 165 ($C_9H_{11}NO_2$; found 165.0791, calculated 165.0790), 149, 147, 132, 123, 108 (base), 95, 80, 65. Comparison of an authentic sample of N-acetyl-p-anisidine with compound 6 showed identical melting point, mixed melting point, retention time and mass spectra.

EXTRACTION AND ISOLATION OF CRUDE BASES OF SHEPHERDIA CANADENSIS

The bark of roots (610 g) were extracted by the procedure reported for S. argentea, yielding 2.2 g crude bases (0.36%). The crude bases were acetylated in the usual manner and chromatographed over Woelm alumina, activity 3. Six major fractions were isolated.

	Eluent	
1	benzene	Simple acetylated bases (4.5%)
2	benzene	Antioxidant (0.5%)
3	benzene	Artifact of extraction (9.5%)
4	benzene-chloroform (5:1)	N,O-diacetyltetrahydroharmol (35%)

5		N,O-Diacetylshepherdine
6	Chloroform methanol (100:1)	N,O-Diacetylserotonin (17%)

N,N´-DIACETYL-N,N´-DI-o-TOLYL-1,2-DIAMINOETHANE (7)

Compound 7 was isolated by elution chromatography on deactiviated alumina using benzene. Compound 7 crystallizes from acetone, mp 148-150° C.

ORD spectrum: no absorption

UV spectrum: λ_{max}^{MeOH} 260 mμ, 270 mμ.

IR spectrum: $\nu_{max}^{CCl_4}$ 1665 cm^{-1} (C=O of an amide), 1600, 1500 cm^{-1}.

NRM spectrum: τ2.74, multiplet of 8 protons (Ar-H); 5.63, multiplet (H-1e,H-2e); 6.83, multiplet (H-1a,H-2a); 7.79, singlet of six protons (N-CO-CH_3); 8.39 doublet of six protons (Ar-CH_3). Addition of D_2O shows no change in the spectrum. Spin-spin decoupling experiments show that the methylene protons are coupled but that the aromatic methyl protons are not coupled to any other proton. Temperature controlled experiments in pyridine-d_5 with HMDS internal standard shows the presence of rotamers.

signal	temperature		assignment
	room	90° C	
τ7.97	doublet	singlet	(N-CO-CH_3)
τ8.38	doublet	broad singlet	(Ar-CH_3)

Mass spectrum: m/e 324 ($C_{20}H_{24}N_2O_2$; found 324.1837, calculated 324.1837), 175, 162, 149, 133, 120, 118, 91.

REDUCTION OF COMPOUND 7

Compound 7 (0.007 g) in ether (5 ml) was added to lithium aluminum hydride (0.060 g) in ether; the mixture was allowed to stand at room temperature 15 minutes, then water (1 ml) and 10% aqueous sodium hydroxide (1 ml) added cautiously. The precipitate was filtered and the filtrate dried (anhydrous magnesium sulfate) and excess solvent removed *in vacuo*. The reaction product (8) had the following spectral properties:

UV spectrum: λ_{max}^{MeOH} 250 mμ; $\lambda_{max}^{H^+}$ 262 mμ, 270 mμ

Mass spectrum: m/e 296 ($C_{20}H_{28}N_2$; found 296.2250), calculated 296.2252), 148, 120, 91.

The UV spectrum of N,N-dimethyl-*o*-toluidine (λ_{max}^{MeOH} 243 mμ; $\lambda_{max}^{H^+}$ 260 mμ, 268 mμ) was very similar to that of compound 8.

SYNTHESIS OF N,N´-DIACETYL-N,N´-DI-*o*-TOLYL-1,2-DIAMINOETHANE (7)[38]

o-Toluidine (11 g) made into a paste with sodium carbonate was heated in a 3-necked flask equipped with a reflux condenser and ethylene dibromide (10 g) was added slowly with stirring. During the process, more sodium carbonate was added, then the temperature was raised to 160°C for 1/2 hour. The residue was washed with water and

o-toluidine removed by steam distillation into glacial acetic acid. Steam distillation was discontinued when oil droplets gathered on the surface of the distillate and the aqueous oily residue was transferred to a separatory funnel and the aqueous layer removed. The residue was crystallized from hot ethanol, mp 161°C, reported 157°C.[38] The product was acetylated in the usual manner, giving N,N´-diacetyl-N,N´-di-o-tolyl-1,2-diaminoethane (7). Compound 7 crystallizes from acetone, mp 148 - 150 C. Its TLC, infrared spectrum and NMR spectrum are identical with that of the naturally occurring compound.

7-ACETOXY-2-ACETYL-1,1-DIMETHYL-1,2,3,4-TETRAHYDRO-2-CARBOLINE (9)

Compound 9 was isolated by elution chromatography over deactivated alumina using benzene. Compound 9 crystallizes from methanol, mp 212 - 214° C.

UV spectrum: λ_{max} 227 mμ, (ε=10,300); 291 mμ, (ε=2,000)

IR spectrum: ν_{max} 3455, 3300 cm^{-1} (indole N-H); 1750 cm^{-1} (C=O) of an ester); 1640 cm^{-1} (C=O of an amide).

NMR spectrum: τ1.58, singlet (N-H); 2.60, doublet J=8 cps (H-5); 2.94, doublet J=2 cps (H-8); 3.20, doublet of doublets J=2,8 cps (H-6); 6.36, triplet (H-3e,3a); 7.22, triplet (H-4e,4a); 7.68, singlet (N-CO-CH_3); 7.75, singlet ($OCOCH_3$); 8.16, singlet ($C(CH_3)_2$). Assignments have been verified by spin-spin decoupling experiments.

Mass spectrum: m/e 300 ($C_{17}H_{20}N_2O_3$; measured 300. 1475, calculated 300.1474), 285, 258, 243 (base), 201, 200.

SYNTHESIS OF 2-ACETYL-1,1-DIMETHYL-1,2,3,4,-TETRAHYDRO-2-CARBOLINE (12).[40]

Tryptamine (0.9 g), acetone (3.5 ml), and p-toluene-sulfonic acid (catalytic amount) in anhydrous benzene (30 ml) were refluxed under nitrogen for 3.5 hours with azeotropic distillation of water. The solution was cooled, filtered over anhydrous potassium carbonate, and excess solvent removed *in vacuo*. The residue was suspended in anhydrous benzene and freshly distilled phosphorus oxychloride (2.5 ml) added. The mixture was allowed to stand at room temperature one hour, then refluxed one hour. Excess solvent was removed *in vacuo* under nitrogen. The residue was heated briefly with water (30 ml), filtered, decolorized with Norit, basified (pH 9) with ammonia, then extracted with ether. The ether fraction was dried (anhydrous magnesium sulfate) and excess solvent removed *in vacuo* to give 1,1-dimethyl-1,2,3,4-tetrahydro-2-carboline (11). Compound 11 was purified by elution chromatography on Woelm alumina, activity 3. Compound 11 crystallizes from benzene, mp 94 - 96°C, reported 111.5 - 115.5° C[40]

NMR spectrum: τ2.20 singlet (N-H); 2.90, multiplet (4 Ar-H); 6.80, triplet (H-3e,3a); 7.30, triplet (H-4e,4a); 8.00,

singlet (N-H); 8.54, singlet (C-$(CH_3)_2$).

Compound 11 was acetylated in the usual manner giving 2-acetyl-1,1-dimethyl-1,2,3,4-tetrahydro-2-carboline, 12. Compound 12 crystallizes from acetone-water, mp 228 - 229°C. NMR spectrum : τ1.67, singlet (N-H); 2.72, multiplet (4 Ar-H); 6.30, triplet (H-3e,3a); 7.16, triplet (H-4e,4a); 7.70, singlet ($NCOCH_3$); 8.09, singlet (C-$(CH_3)_2$).

EXTRACTION AND ISOLATION OF BASES OF *SHEPHERDIA CANADENSIS* USING REAGENT METHANOL.

Dried, ground roots (710 g) of *S. canadensis* were extracted with reagent methanol (free from acetone) in a Soxhlet extractor. Excess solvent was removed in vacuo, water added and traces of methanol removed. The aqueous extract was acidified (pH3) with 5% aqueous hydrochloric acid, then extracted with ether to remove neutral and acidic material. The aqueous acidic fraction was basified (pH9) with sodium carbonate, the precipitate filtered, then continuously extracted with ether for 48 hours. The ether fraction was dried (anhydrous magnesium sulfate) and excess solvent removed *in vacuo* yielding crude bases (3.3 g).
The crude bases (1.3 g) isolated by extraction of *S. canadensis* with reagent methanol were acetylated in the usual manner yielding crude acetylated bases (2.1 g).
Mass spectrum: m/e 286, 260, 229, 175.
Elution chromatography of the crude acetylated bases on

Woelm alumina, activity 3 gave no trace of compound 9.

ISOLATION OF N,O-DIACETYLTETRAHYDROHARMOL (1) FROM *SHEPHERDIA CANADENSIS*

N-O-diacetyltetrahydroharmol was isolated by elution chromatography of the crude acetylated bases of *S. canadensis* on deactivated alumina using benzene-chloroform (5:1). It was separated from N,O-diacetylshepherdine by fraction crystallization from methanol. It has a melting point 202° C and its spectral properties are identical with those reported for compound 1.

SYNTHESIS OF N,O-DIACETYLTETRAHYDROHARMOL, (1)[35]

Sodium (1.5 g) was added to a refluxing solution of harmol ((13)(0.1 g) Aldrich Chemical Co) in anhydrous ethanol under a nitrogen atmosphere. When all the sodium was dissolved, the solution was acidified (aqueous HCl), the precipitate filtered and washed with ethanol. The ethanol washings and filtrate were combined and excess solvent removed *in vacuo*. The crude residue was dissolved in water, the solution basified with aqueous ammonia, then extracted with ether. The ether was dried (anhydrous magnesium sulfate), then removed *in vacuo* to give tetrahydroharmol (3). Tetrahydroharmol was acetylated in the usual manner and the crude acetate purified by elution chromatography over Woelm alumina, activity 3. N,O-diacetyltetrahydroharmol crystal-

lizes from ethyl acetate, mp 202° C. Its spectral properties are identical with those of the naturally occurring alkaloid.

N,O-DIACETYLSHEPHERDINE (2)

N,O-diacetylshepherdine was isolated by elution chromatography of the crude acetylated bases of S. canadensis on deactivated alumina using benzene-chloroform (5:1). It was separated from N,O-diacetyltetrahydroharmol by fractional crystallization from methanol. It crystallizes from methanol, mp 192 - 194°C and gives a depressed mixed melting point with N,O-diacetyltetrahydroharmol, mp 165 - 175°C.

UV spectrum: λ_{max} 226 mμ, (ε=31,400), 281 mμ (ε=6,300).

IR spectrum: ν_{max} 3450, 3275 cm^{-1} (indole N-H); 1745 cm^{-1} (C=O of an ester); 1630 cm^{-1} (C=O of an amide).

NMR spectrum: τ1.14, singlet (N-H); 2.80, doublet J= 8 cps (H-8); 2.92, doublet J= 1 cps (H-5); 3.22, doublet of doublets J= 1,8 cps (H-7); 4.30 quartet J= 6 cps (H-1); 6.07, multiplet (H-3e), 6.64, multiplet (H-3a); 7.34, multiplet (H-4e,4a); 7.71, singlet ($NCOCH_3$); 7.80, singlet ($OCOCH_3$); 8.62, doublet, J=6 cps ($C-CH_3$). Assignments have been verified by spin-spin decoupling experiments.

Mass spectrum: 286 ((base) $C_{16}H_{18}N_2O3$; found 286.1317, calculated 286.1317), 271, 244, 229, 201, 187.

SYNTHESIS OF N,O-DIACETYLSHEPHERDINE (2)[44]

5-Benzyloxytryptamine ((0.1 g) Aldrich Chemical Co), 2N sulfuric acid (5 drops), dioxane (1 ml) and water (1 ml) were cooled under nitrogen and freshly prepared 10% aqueous acetaldehyde (2 ml) added. The mixture was allowed to stand at room temperature. After 0.5 hour a crystalline precipitate formed which was collected and acetylated in the usual manner to give 2-acetyl-6-benzyloxy-1-methyl-1,2,3,4-tetrahydro-2-carboline (15). Compound 15 crystallizes from acetone-methanol, mp 179.5 - 181°C. Compound 15 (0.4 g) was hydrogenated with 30% palladium-charcoal (catalytic amount) in reagent methanol (15 ml) at room temperature and atmospheric pressure for 4 hours, i.e., until starting material had all reacted (TLC). The solution was filtered, excess solvent removed in vacuo and the crude residue acetylated in the usual way. The crude acetyl derivative was purified by elution chromatography on Woelm alumina, activity 3, to give 6-acetoxy-2-acetyl-1-methyl-1,2,3,4-tetrahydro-2-carboline (2) crystallizes from methanol, mp 192 - 194°C. Its melting point, mixed melting point and infrared spectrum are identical with that of the naturally occurring N,O-diacetylshepherdine.

N,O-DIACETYLSEROTONIN (16)

N,O-diacetylserotonin was isolated by elution chromatography of the crude acetylated bases of S. canadensis

over deactivated alumina using chloroform. N,O-diacetylserotonin could not be crystallized.

UV spectrum: λ_{max} 224 mμ, (ε=27,000), 287 mμ (ε=5,500).

IR spectrum: ν_{max} 3500, 3325 cm^{-1} (indole N-H); 1750 cm^{-1} (C=O of an ester); 1675 cm^{-1}(C=O of an amide).

NMR spectrum: (acetone-d_6) τ -0.20, singlet (N-H); 2.68, doublet J= 8 cps (H-7); 2.75, doublet J= 2 cps (H-4); 2.86, singlet (H-1), 3.21, doublet of doublets J=2,8 cps (H-6); 6.58, multiplet (H-2´e,2´a); 6.77, broad singlet ($NHCOCH_3$); 7.15, triplet (H-1´e,1´a); 7.79, singlet ($NCOCH_3$); 8.15, singlet ($OCOCH_3$). Assignments made have been verified by spin-spin decoupling experiments. In addition, irradiation at 1020 cps causes sharpening of signals at τ2.75 and 2.86 indicating a small W coupling between the indole N-H and H-4, and a small coupling between indole N-H and H-1. Irradiation at 716 cps causes sharpening of the signal at τ3.21 showing a small para coupling between H-4 and H-7.

Mass spectrum: m/e 260 ($C_{14}H_{16}N_2O_3$; found 260.1158, calculated 260.1161), 201, 188, 159, 146.

SYNTHESIS OF N,O-DIACETYLSEROTONIN (16)

Serotonin creatinine sulfate (Aldrich Chemical Co) 9.1 g) was acetylated in the usual manner. Creatinine was removed by addition of acetone and filtering the precipitate. Excess acetone was removed *in vacuo* and the crude N,O-diacetylserotonin purified by elution chromatography

on Woelm alumina, N,O-diacetylserotonin has an infrared spectrum identical with that of the naturally occurring compound.

EXTRACTION OF CLEMATIS LIGUSTICIFOLIA

Dried, ground roots (2600 g) were extracted with 5% aqueous methanol in a Soxhlet extractor. A flocculent precipitate was filtered from the cooled methanol extract and excess methanol was removed *in vacuo*. The tar-like residue was extracted with acetone in a Soxhlet extractor, then excess acetone removed *in vacuo* yielding acetone-soluble material (37.4 g). The acetone-soluble material was dissolved in chloroform-*t*-butyl alcohol (20:1), then separated into basic, strong and weak acidic and neutral fractions in the following manner. The chloroform solution was extracted with 5% aqueous hydrochloric acid. The acid fraction was basified (pH 10) with potassium carbonate, then extracted with chloroform. The chloroform fraction was dried (anhydrous magnesium sulfate), then excess solvent removed *in vacuo* yielding crude bases (0.1 g). The chloroform-*t*-butyl alcohol fraction was extracted with 5% aqueous potassium hydroxide. The basic fraction was acidified with hydrochloric acid, extracted with chloroform, the chloroform fraction dried (anhydrous magnesium sulfate), then excess solvent removed *in vacuo* yielding strong and weak acids (17.0 g). The chloroform-*t*-butyl alcohol fraction

from which the basic and acidic material had been removed was dried (anhydrous magnesium sulfate), then excess solvent removed *in vacuo* yielding neutral material (12.6 g).

β-SITOSTEROL (19)

β-Sitosterol was isolated by elution chromatography of the neutral material of *C. ligusticifolia* on silicic acid using chloroform-methanol (100:1). β-Sitosterol crystallizes from methanol-acetone, mp 136 - 138°C.

IR spectrum: ν_{max} 3615, 1150 cm^{-1} (O-H)

Mass spectrum: m/e 414, 396

β-Sitosterol was acetylated in the usual manner yielding O-acetyl-β-sitosterol. O-Acetyl- β-sitosterol crystallizes from methanol, mp 134° C (reported 134° C).[48]

A comparison of an authentic sample of β-sitosterol with compound 19 showed identical melting point (135 - 136° C), mixed melting point (135 - 136° C) and IR spectra.

4,7-DIMETHOXY-5-METHYLCOUMARIN (20)

Compound 20 was isolated by elution chromatography of the neutral material of *C.ligusticifolia* on silicic acid with chloroform-methanol (50:3). Compound 20 crystallizes from methanol, mp 185 - 187° C; repeated crystallization 190 - 191.5° C.

UV spectrum: λ_{max} 308 mμ(ε=13,000)

IR spectrum: ν_{max} 1700 cm^{-1} (C=O of an α,β-unsaturated

system);1600, 1475 cm^{-1} (aromatic C=C stretch).
NMR spectrum: τ3.36, doublet J= 2.8 cps (Ar-H); 3.42, doublet J= 2,8 cps (Ar-H); 4.49, singlet (C=C-H); 6.08, singlet ($C=C-OCH_3$); 6.18, singlet ($Ar-OCH_3$); 7.40, singlet ($Ar-CH_3$).
Mass spectrum: m/e 220 ($C_{12}H_{12}O_4$; found 220.1740, calculated 220.0749), 192, 177, 149.

SYNTHESIS OF 4,7-DIMETHOXY-5-METHYLCOUMARIN (20)

Anhydrous hydrogen chloride was bubbled into a solution of orcinol (12 g), ethyl cyanoacetate (13 g) and zinc chloride (6 g) in anhydrous ether (90 ml). Excess solvent was removed and the crude oil poured onto ice. The resultant precipitate was filtered and heated on a steam bath with hydrochloric acid:water (1:1) for 1.5 hours. The crystalline product, 4,7-dihydroxy-5-methylcoumarin (22) crystallizes from hot water (mp 272 - 274° C, reported 266 - 267°C [52]). Compound 22 (2 g) was dissolved in 10% aqueous potassium hydroxide (15 ml) and dimethyl sulfate (15 g) added and left at room temperature overnight. The precipitate, 4,7-dimethoxy-5-methylcoumarin (20) was filtered and crystallized from hot methanol, mp 185 - 187°C. Compound 20 was purified by repeated crystallization from methanol. Its melting point (190 - 191.5°C), mixed melting point (190 - 191.5°C) and spectra were identical with that of the naturally occurring compound.

SYNTHESIS OF 5,7-DIMETHOXY-4-METHYLCOUMARIN (21)

Diketene (8.5 g) was added dropwise during 3/4 hour to a mixture of 3,5-dimethoxyphenol (prepared by the method of Stork and Tomasz)[59], (11.5 g), and triethylamine (0.5 cc). The mixture was heated 0.5 hours, cooled then added dropwise with agitation to warm (70 - 80° C) concentrated sulfuric acid (15 ml). After addition was completed, the mixture was heated 80 C) for 1 hour, then cooled and poured slowly onto ice. The resultant precipitate was filtered giving 5,7-dimethoxy-4-methylcoumarin (21). Compound 21 crystallizes from methanol (mp 173 - 175° C, reported 179 - 180° C [57]).

UV spectrum: λ_{max} 320 mμ (ε=18,400).

IR spectrum: ν_{max} 1710 cm^{-1} (α,β-unsaturated C=O); 1600, 1475 cm^{-1} (aromatic C=C stretch).

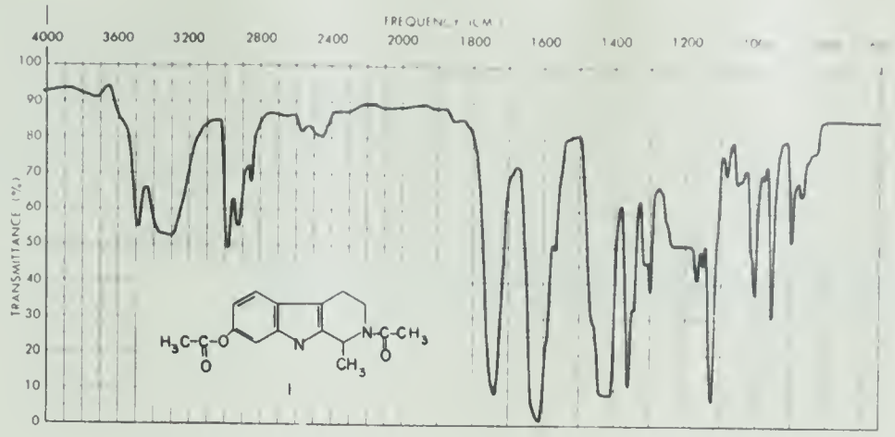
NMR spectrum: (A-60 model τ3.60, doublet J=2,5 cps (Ar-H); 3.70, doublet J= 2.5 cps)(Ar-H); 4.08, doublet J= 1 cps (C=C-H); 6.18, singlet of six protons (Ar-OCH_3); 7.48, doublet J= 1 cps (C=C-CH_3).

Mass spectrum: m/e 220 ($C_{12}H_{12}O_4$ found; 220.0735, calculated 220.0736), 192, 177, 149.

A mixed melting point of compound 21 with that of the naturally occurring coumarin was depressed, mp 148 - 150° C.

EXTRACTION OF SILICONE GREASE

Silicone grease ((1 g), Dow Corning) in reagent methanol (50 ml) was caused to reflux overnight. The methanol was decanted from excess grease and excess methanol removed *in vacuo*. The residue remaining was acetylated in the usual manner yielding acetylated material (0.120 g). The acetylated material was chromatographed over Woelm alumina, activity 3. One fraction, which was eluted with benzene, had a compound (TLC, mixed TLC) identical to that of N,N´-diacetyl-N,N´-di-*o*-tolyl-1,2-diaminoethane.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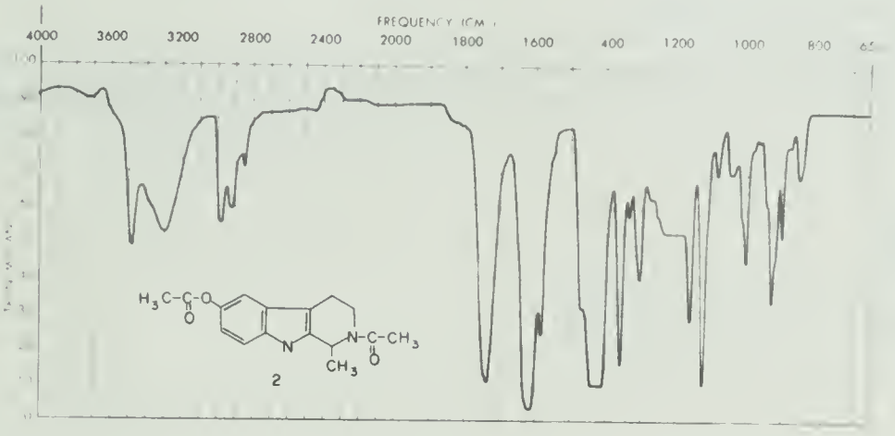
N
N-C-CH3
O
CH3

1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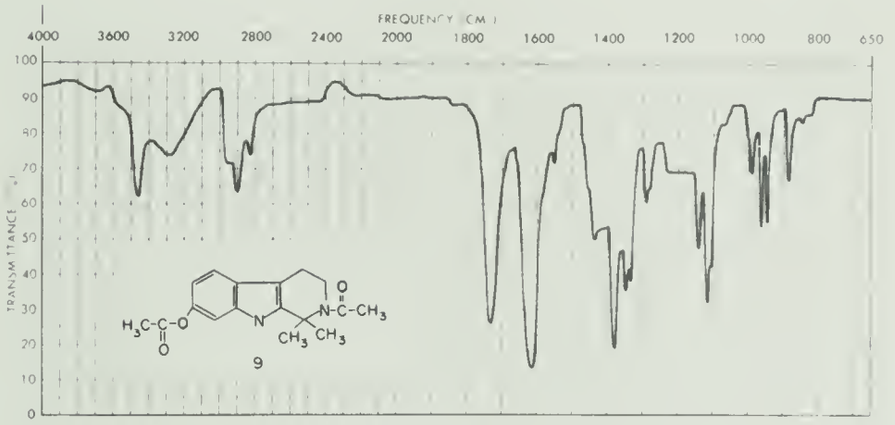
O
N
N-C-CH3
O
CH3

2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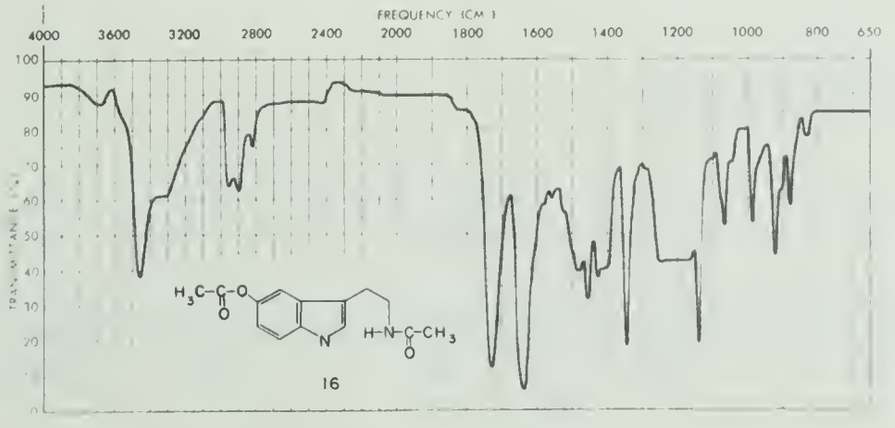
O
N-C-CH3
CH3 CH3

9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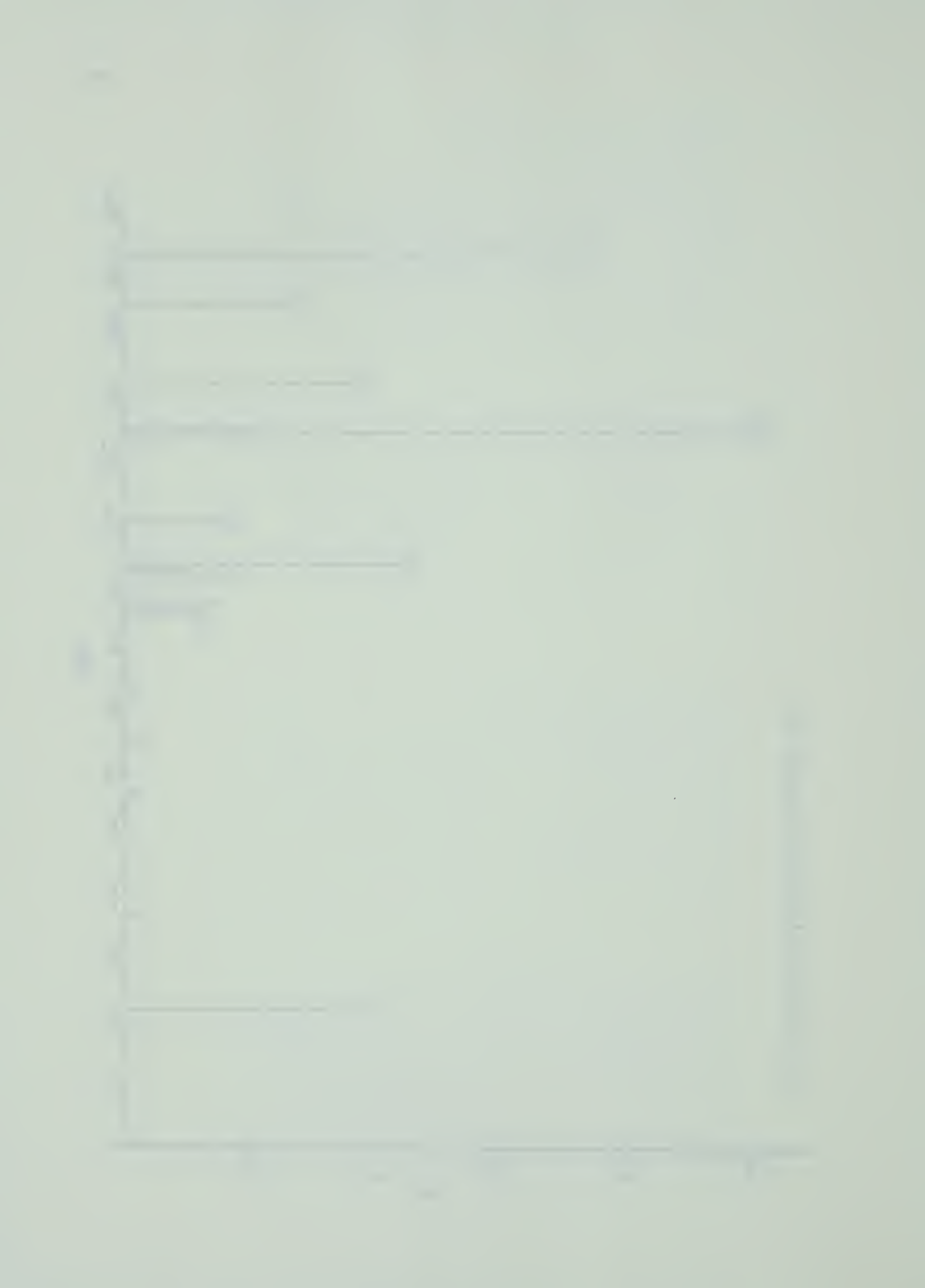
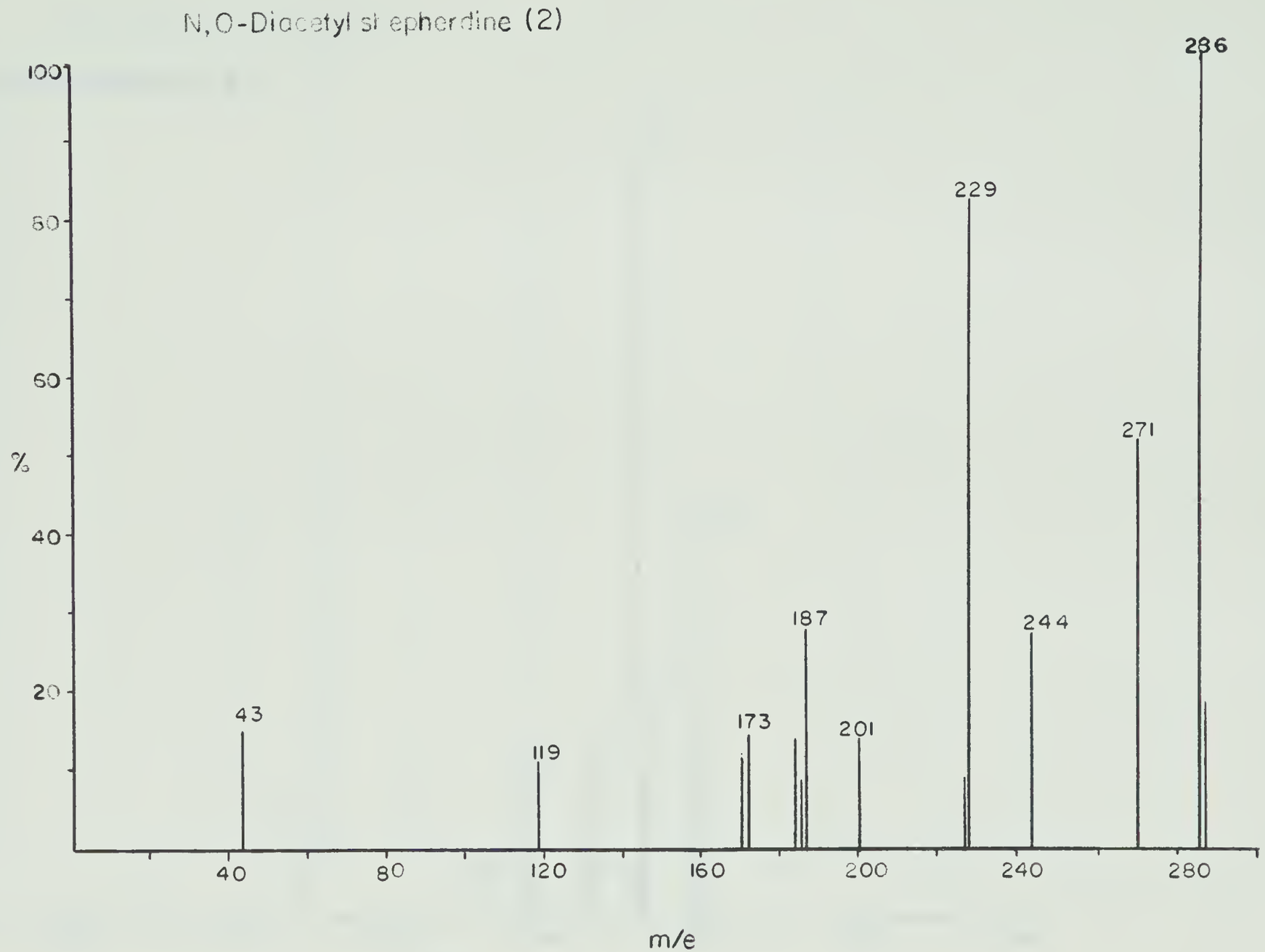
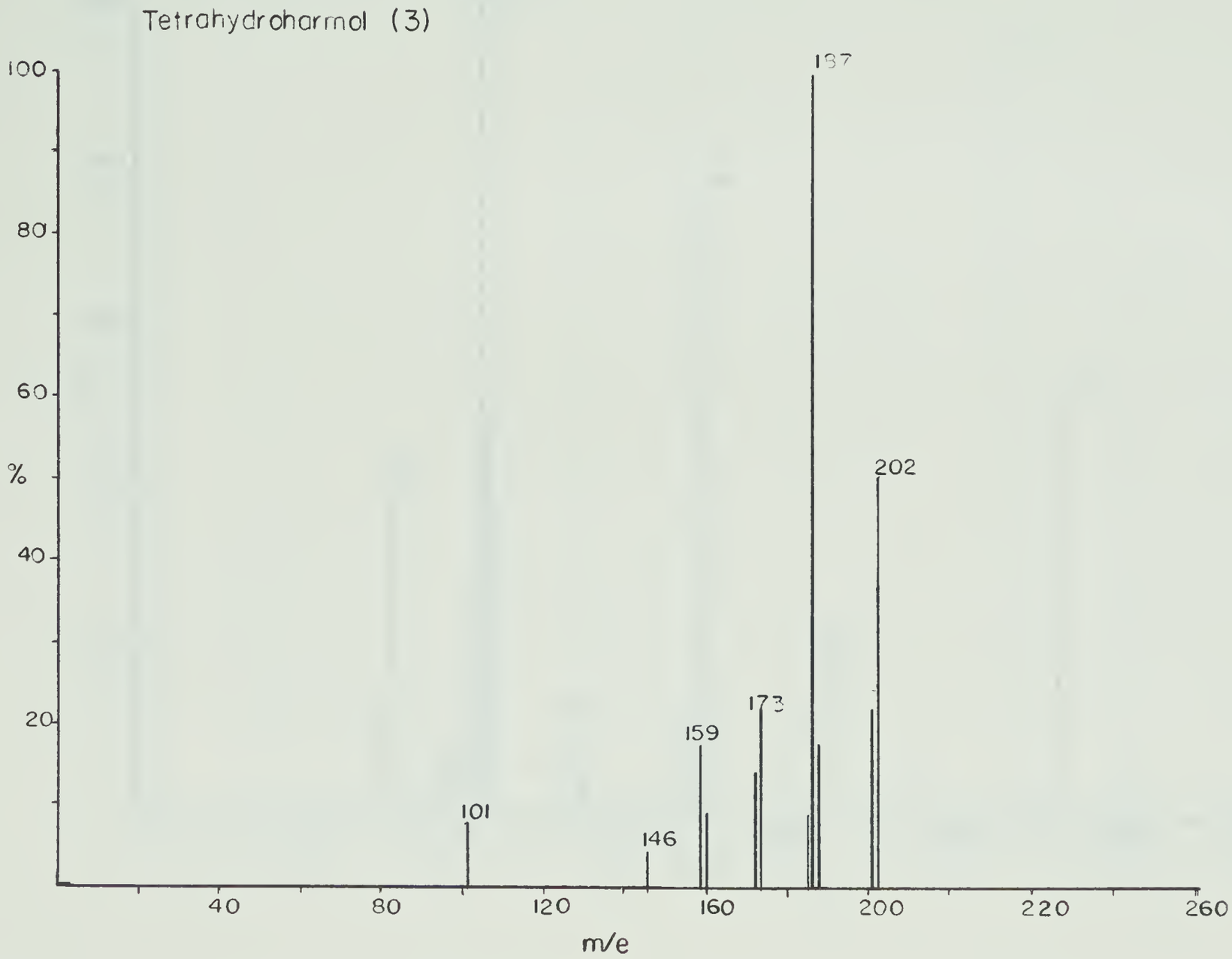
100 90 80 70 60 50 40 30 20 10 0
H3C-C-O
O
N
H-N-C-CH3
O

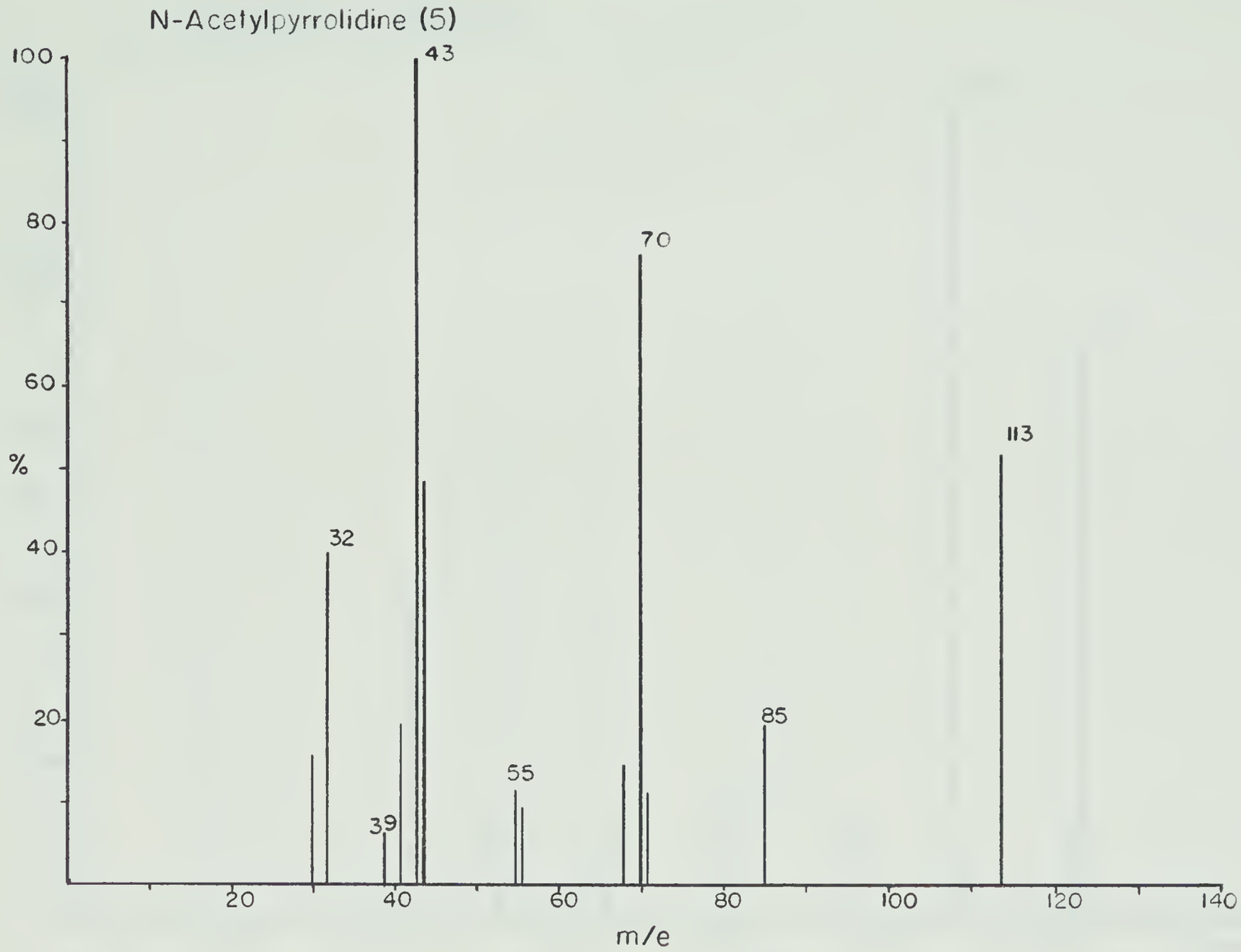
16

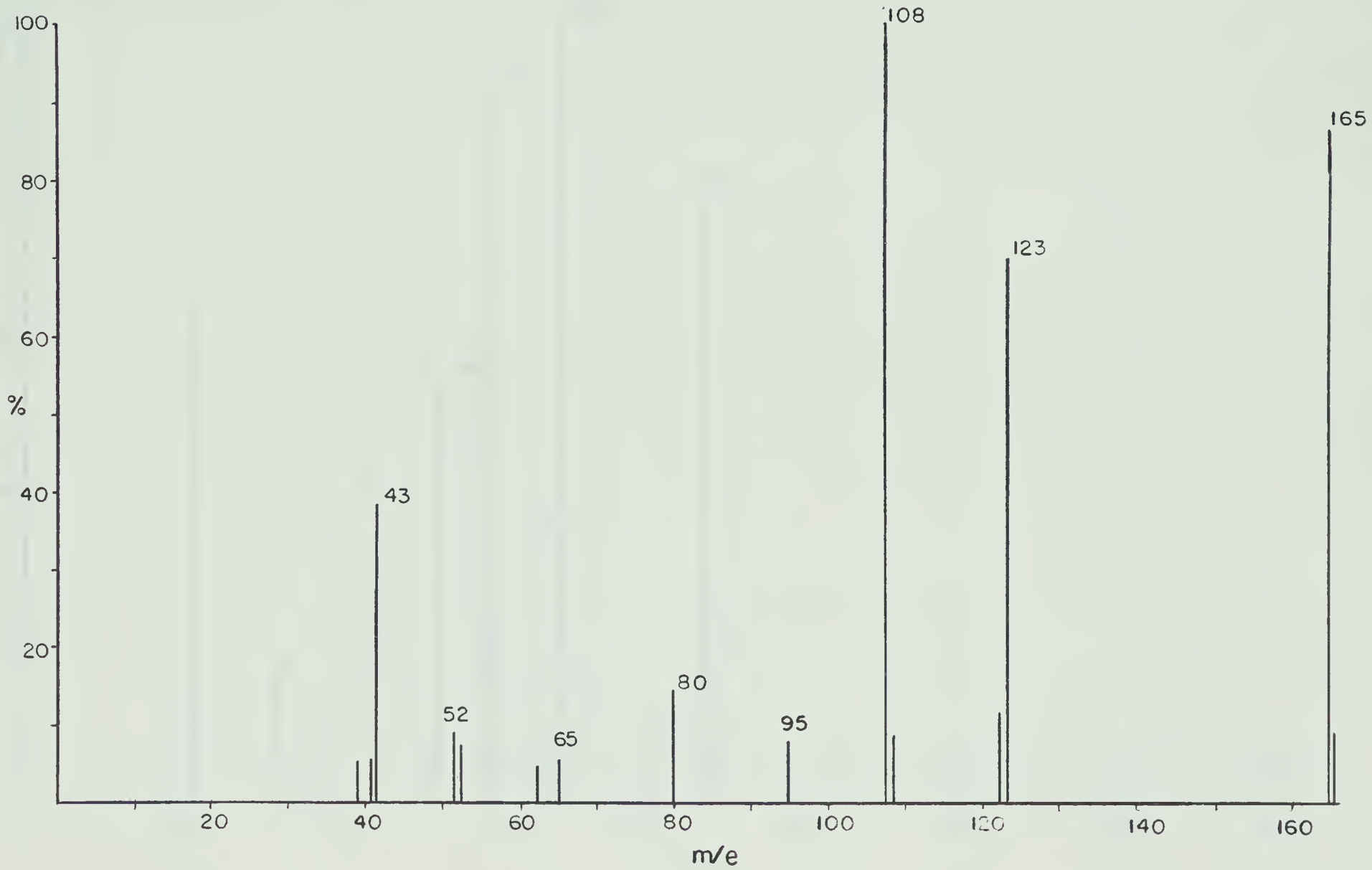

N, O - Diacetyl tetrahydronarmol (I)
%
m/e
100
80
60
40
20
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
43
172
187
201
229
244
271
286

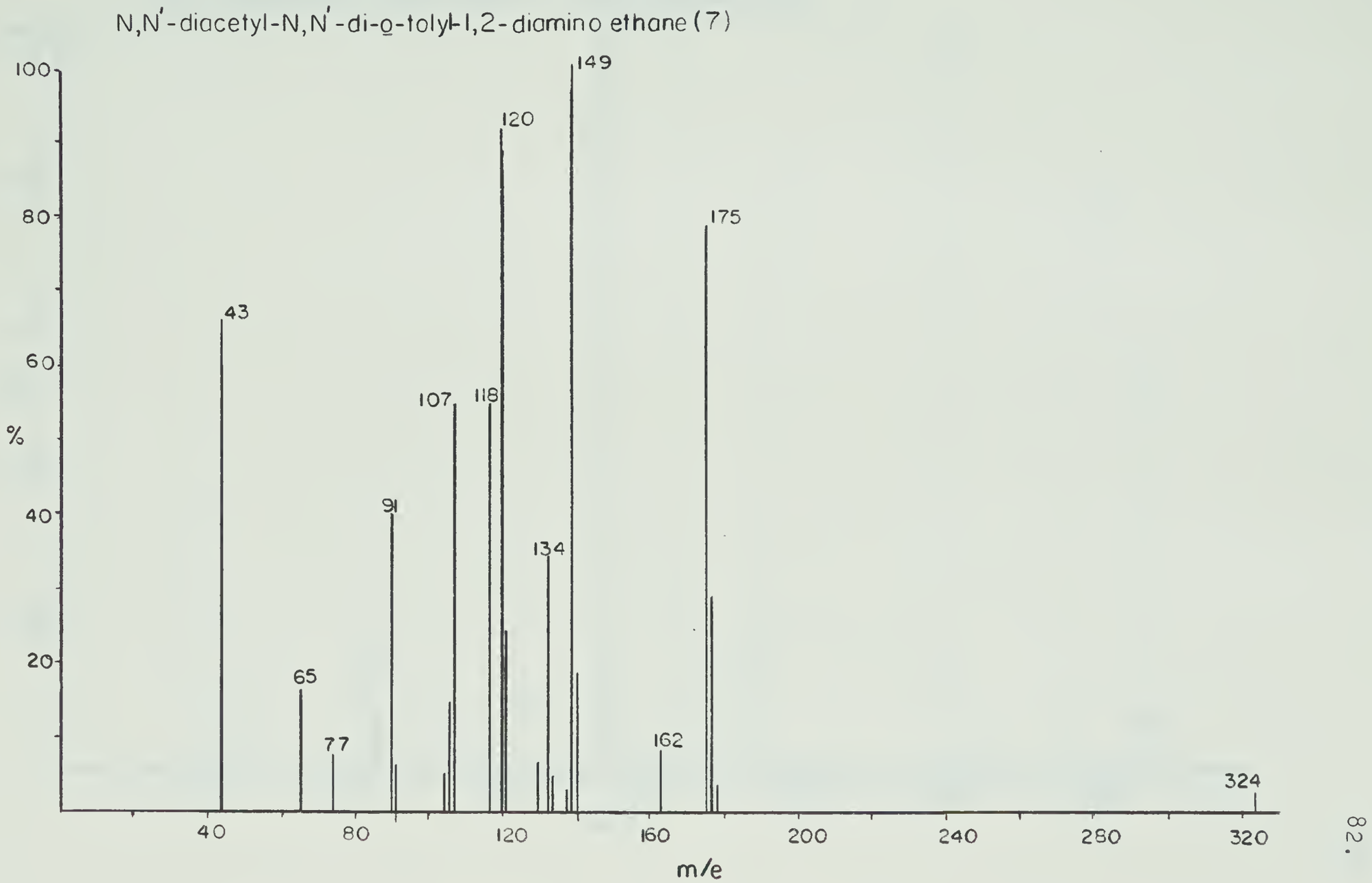



N,O-Diacetyl sl epherdine (2)
100
80
60
40
20
%
286
229
271
244
187
173
201
119
43
40
80
120
160
200
240
280
m/e

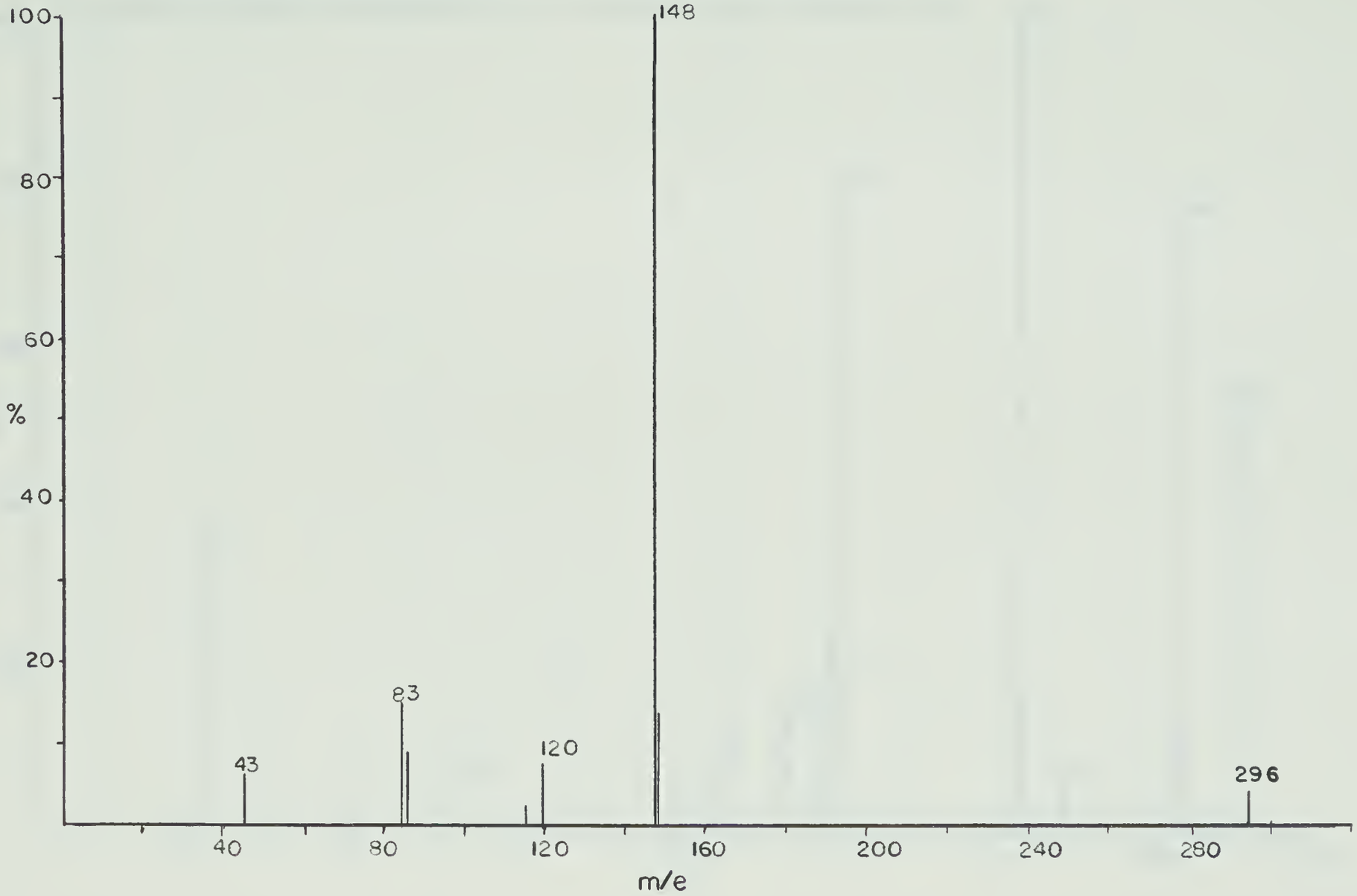

Tetrahydroharmol (3)
100
80
60
%
40
20
137
202
173
159
101
146
40
80
120
160
200
220
260
m/e

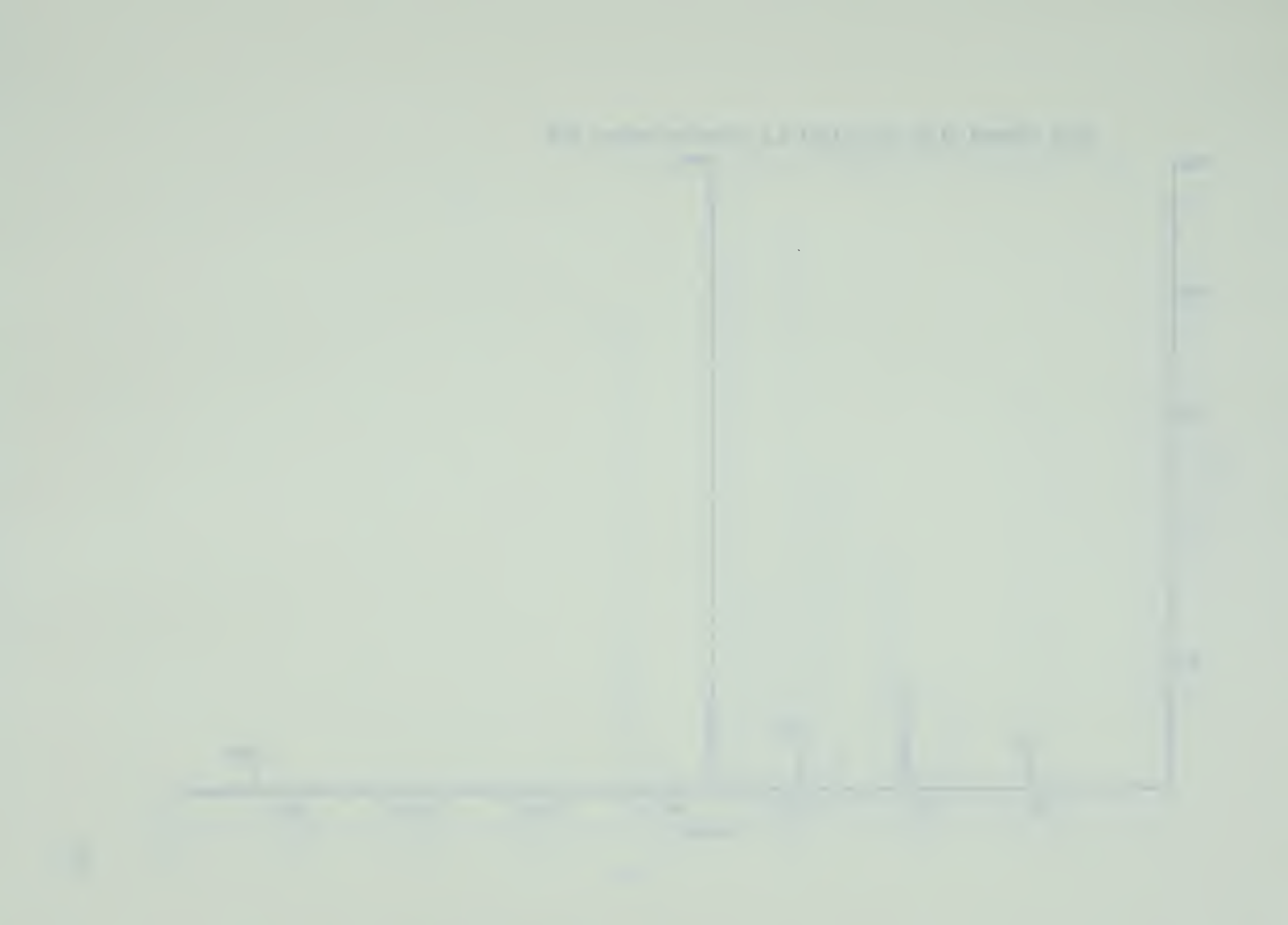

N-Acetylpyrrolidine (5)
100
80
60
40
20
%
43
70
113
32
85
55
39
20
40
60
80
100
120
140
m/e

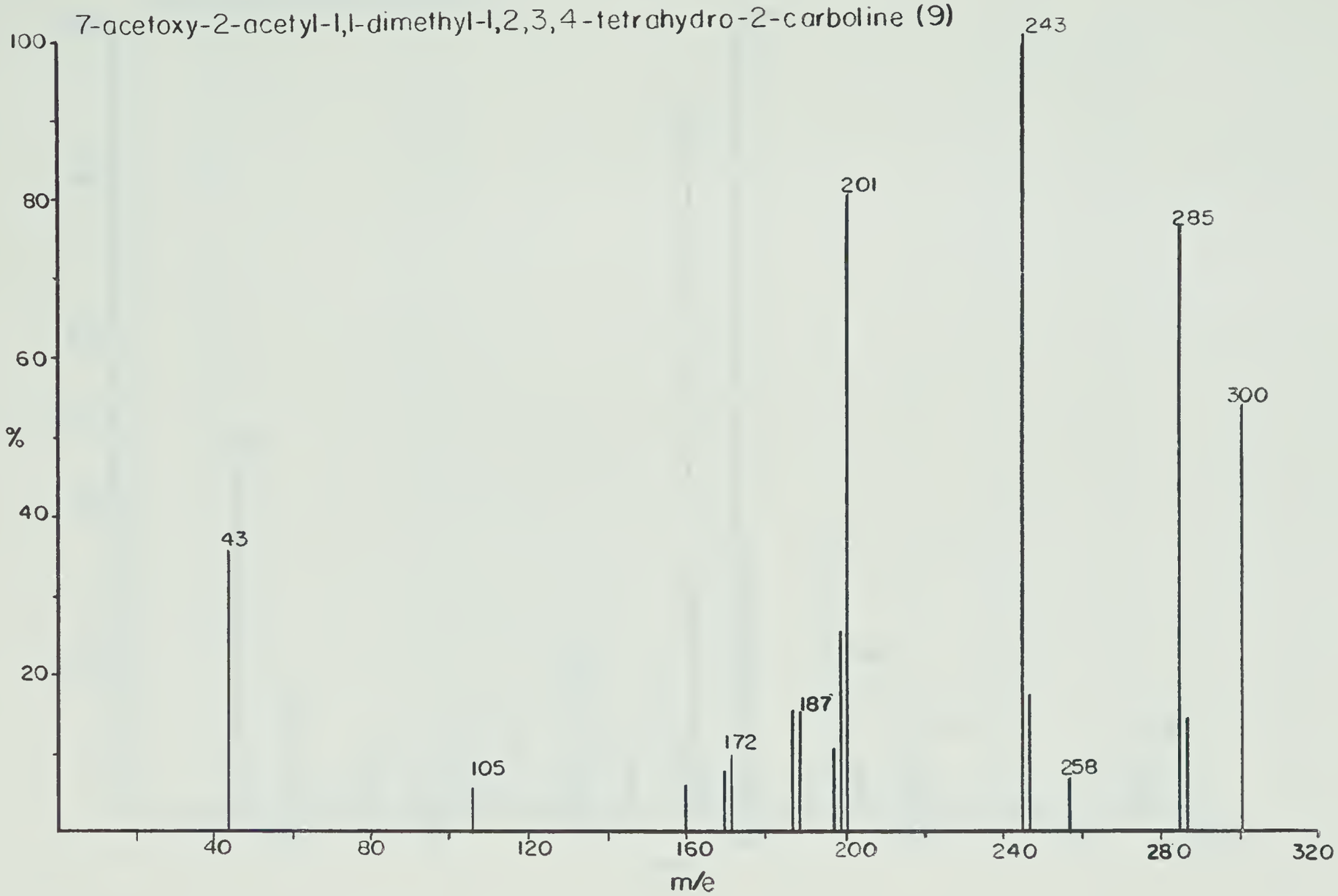

N-acetyl-p-anisidine (6)
100
80
60
%
40
20
108
165
123
43
52
65
80
95
20
40
60
80
100
120
140
160
m/e


N,N'-diacetyl-N,N'-di-o-tolyl-1,2-diamino ethane (7)
100
80
60
40
20
%
149
120
175
43
107
118
91
134
65
77
162
324
40
80
120
160
200
240
280
320
m/e

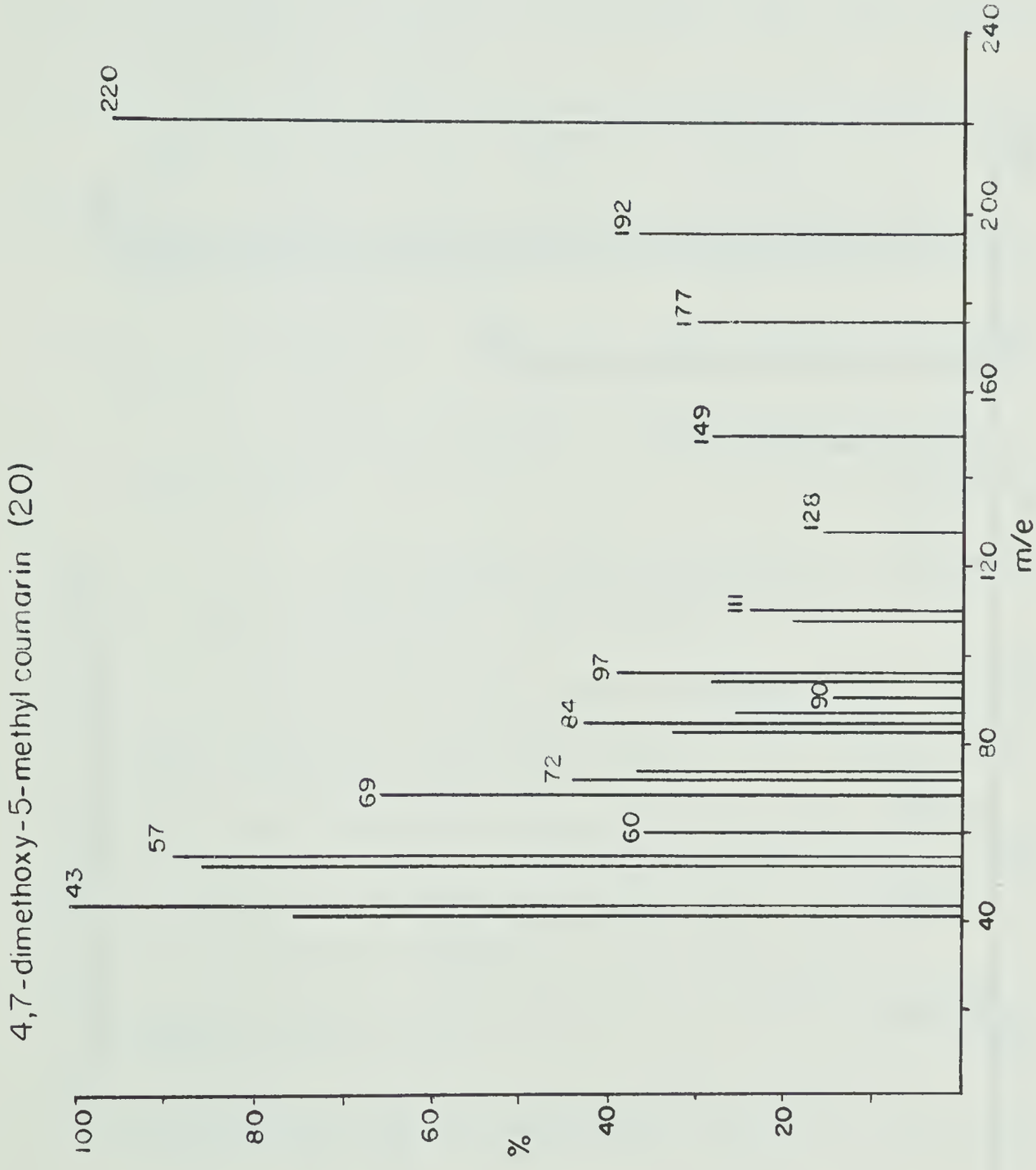
N,N -diethyl-N,N -di-o-tolyl-1,2 -diamino ethane (8)

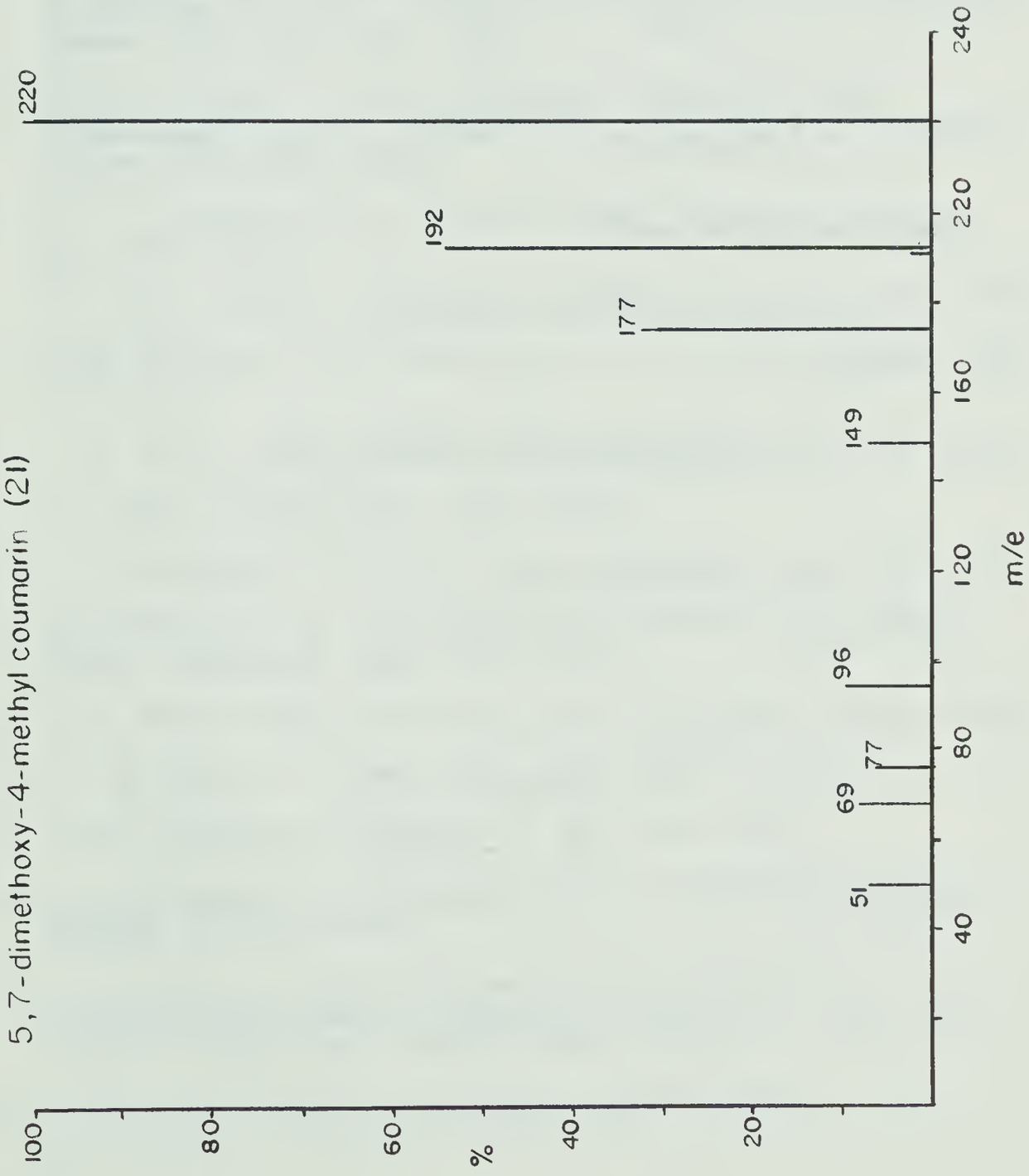





7-acetoxy-2-acetyl-1,1-dimethyl-1,2,3,4-tetrahydro-2-carboline (9)
100
80
60
40
20
%
243
201
285
300
43
105
172
187
258
40
80
120
160
200
240
280
320
m/e


N,O-diacetylserotonin (16)
100
80
60
40
20
%
159
146
30
43
65
91
117
130
188
202
260
40
80
120
160
200
240
280
m/e


4,7-dimethoxy-5-methyl coumarin (20)
100
80
60
40
20
%
43
57
69
60
72
84
97
90
111
128
149
177
192
220
40
80
120
160
200
240
m/e


5,7-dimethoxy-4-methyl coumarin (21)
100
80
60
%
40
20
220
192
177
149
96
77
69
51
40
80
120
160
220
240
m/e

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